University of Alberta

The Inter-relationship between Enamel Chemical Composition, *in vitro* Bond strength and Qualitative Etching Pattern

by

Dr. Leo Lou ©

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This thesis is dedicated to

Lou Chi Chung, my grandfather.

A cast-iron teddy-bear, whose life was an example of the virtues of dedication and perseverance in everything he pursued… and most of all, in the way he looked after the people he loved.

And

William and Angela Lou, my parents.

My mentors, my compass and my best friends, from whom I have learned the secret to a full and happy life:

Try many new things, to find those you like.
Repeat those you like, to determine what you love.
Practice what you love, to discover your life's passions.
Pursue your life's passions, each and everyday…
and always keep trying many new things.

ABSTRACT

Objective: To determine if a difference exists in the chemical composition of buccal enamel surface of maxillary right and left human first premolars and the inter-relationship between chemical compositions, *in vitro* shear bond strength and qualitative etching pattern.

Methods: Buccal enamel chemical compositions of 49 pairs of maxillary first premolars were determined using X-ray photoelectron spectroscopy. The etching pattern was examined using scanning electron microscopy. The *in vitro* shear bond strength was measured using MTS machine.

Results: No significant variability in the chemical compositions was found. Chemical composition was not a significant predictor of *in vitro* shear bond strength and etching pattern. Etching pattern was not a significant predictor of *in vitro* shear bond strength.

Conclusions: Chemical compositions of the right and left maxillary first premolars were not significantly different. Regression analysis indicated no significant relationship between chemical composition, *in vitro* shear bond strength and etching pattern.

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Chapter 1

Introduction

And

Literature Review

1.1 INTRODUCTION

Aligning the teeth with fixed orthodontic appliance to give patients a cosmetically-pleasing smile involves the bonding of brackets to enamel surface through an acid-etch technique with composite adhesive. The bonded brackets serve as a device to mechanically hold the orthodontic wire against the teeth so that controlled force can be applied to result in tooth movement. When brackets fail to bond to the enamel surface, the force exerted onto the teeth is lost and no movement of teeth will take place. To restore the force on the teeth, the patient will need to take time off from work or school to go back to the orthodontist to have the brackets rebonded back onto the tooth. This is not only an inconvenience for the patient but it adds unnecessary expense to the orthodontist in the form of lost chair time. Ultimately when a bond fails, there is economic loss for the patient and orthodontist.

Understanding the causes of bracket failure and conditions which may reduce bond failure, therefore, has a significant financial benefit. Many research studies have examined the various bonding variables such as bonding adhesives,[1] the design of brackets, [2, 3] the etching time [4] and the different techniques of bonding.[5] But bond failure still presents as a major problem, ranging from 0.5% to 16% [6, 7] in the everyday practice of orthodontics. It has been speculated that the surface chemical composition of the enamel can be a contributing factor to bond strength possibly by affecting the quality of the etched enamel. [8, 9] But to date, there has not been not been a published study examining the enamel surface chemical composition using extracted human maxillary teeth from subjects undergoing orthodontic treatment and correlating it to the laboratory bond strength and actual clinical bond failure rate through out the entire duration of orthodontic treatment. The primary focus of the study was to determine if the surface chemical composition of the enamel surface can be a predictor of qualitative etching pattern and *in vitro* bond strength.

1.2 LITERATURE REVIEW

1.2.1 ENAMEL CHEMICAL COMPOSITION

Enamel is the most mineralized and the hardest biological substance of human body. It consists of 96% mineral and 3% organic material and water by weight. The inorganic content of the mineral consists mainly of hydroxyapatite (HA) made of crystalline calcium phosphate ($Ca_5(PO_4)_3(OH)$) of about 30 nm thick by 60 nm wide and several microns long.[10] The remainder of the enamel is made up of around 1 % organic material made of proteins and lipids and 3% water dispersed between the hydroxyapatite crystals. Various ions and trace elements can be incorporated or adsorbed into the HA crystals if present during the stage of enamel formation, a process known as amelogenesis.

The process of enamel matrix formation and calcification during amelogenesis can be divided into several stages.[11] In the proliferative phase, cells of preamlobast form and differentiate into functioning ameloblasts which secret enamel matrix proteins (amelogenins and enamelins) during the presecretory phase. The protein gel adjacent to the ameloblasts is supersaturated in calcium and phosphate ions and carbonated HA is precipitated almost immediately. Once the secretion of the protein by ameloblasts is stopped, the apatite crystals will mature and grow while the proteins will dissolve and resorb. Eventually, the ameloblasts will withdraw and leave apatite crystal stacked in rods with an enamelin-rich boundary between the rods. Once the formation of tooth is completed, the enamel forming cells will be lost and no repair will be possible. Enamel in essence, is a non-vital tissue and is incapable of regeneration.

The mineral making up the inorganic part of human teeth is based primarily on HA. (Fig. 1.1) Pure HA, however, does not occur on a macroscopic scale in biological systems. Human enamel, dentin, cementum and bone are instead composed of a calcium-deficient and carbonate-containing apatite analogue.[12] Carbonate has been shown to substitute for the hydroxyl groups, but this is believed to occur only on a very small scale. Instead, the planar CO_3^{-2} group has been shown to mainly substitute for the tetrahedral PO_4-^{3} which causes the disruption of the HA crystal structure and weakening of the chemical bonds.[13] Charge neutrality is believed to be maintained through calcium deficiency via Ca^{+2} absences or through substitution with Na^{+}.[10]

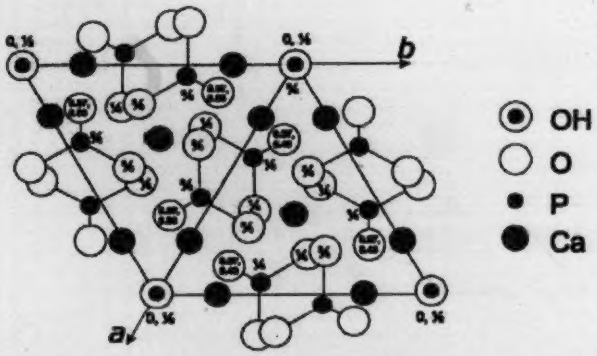


Figure 1.1 The idealized crystal structure of hydroxyapatite (HA), viewed along the c-axis. Biological HA adopts the hexagonal structure with the OH groups ordered along the c-axis. Ca^{2+} ions can occupy two different sites: positions on the corner of two 60° rotated triangles close in to the c-axis and positions at the corner of a hexagonal at a further distance from the c-axis. (Jones FH. Surface Science Reports, 2001:86).

Many other ions and trace elements can also be incorporated into the HA crystal; metal cations can substitute calcium, silicate can substitute phosphate and halide ions can replace hydroxide.[10] The resulting substitution can occur either throughout the entire thickness of enamel apatite crystals or is limited to the surface of the enamel. The type and extent of ion substitution can have a critical effect on the surface chemistry behavior of the enamel.

The distribution within the enamel however, is not the same for all ions. Some ions have higher concentration on the surface of enamel than within, while others have less concentration on the surface than within. Ions which readily become attached to the enamel apatite crystals such as fluoride (F^-) and zinc (Zn) tend to build up in the outer enamel surface which is bathed in tissue fluid after mineralization and before eruption, and in saliva, food and drinks after eruption. On the other hand, ions such as chloride (Cl^-), carbonate (CO_3^-), magnesium (Mg^{+2}) and sodium (Na^+) which readily dissolved out from the calcified tissues by body fluids will have the lowest concentration in the outer enamel.

1) Sodium

There is a steady decline in the sodium content from the surface to the inner enamel. The type of sodium in the enamel is uncertain but some is hypothesized to be adsorbed onto apatite and some within the crystal in exchange with calcium.[14] The sodium concentration of enamel is higher than any other tissue in the body.

2) Chloride

It is generally agreed that there is a steady decline of chloride content from the surface to the inner enamel.[15] Despite the relatively large amounts of Cl^- in biological solutions, Cl^- substituted apatites are not a major constituent of hard tissue.[16]

3) Carbonate

The formation of carbonate is believed to be the result of the metabolic activity of the ameloblasts that form the enamel apatite.[17] When dental enamel is laid down by ameloblasts carbon dioxide is produced. The higher the metabolism of the cells the higher the level of carbon dioxide produced, and the greater the probability of acquiring carbonate into the apatite crystals. As the ameloblasts reach the surface of the enamel, their metabolism slows down and the cells die off. Therefore, it is expected that the concentration of carbonate in enamel should decrease towards the surface, and this decrease has been documented.[18, 19, 20] The carbonate-substituted apatite crystals causes a reduction in crystallinity, which is a reflection of the reduction in apatite crystal size and an increase in crystal strain.[21, 22] The result will be enamel with a greater proportion of weaker and smaller crystals which are more soluble and prone to acid attack. The subsurface of enamel which contains more carbonate than its outer surface, has been shown to have smaller enamel crystals.[23] There is however, a tendency for the carbonate of the outer enamel to fall with age probably as a result of it being gradually dissolved out by acids in the bacterial plaque.[19]

4) Magnesium

The distribution of magnesium has been determined in the enamel of permanent teeth by Robinson *et al.*[24] Magnesium concentration was shown to increase from the surface towards the interior. The distribution pattern of magnesium has been linked to low enamel density and possibly high protein concentration. Enamel with relatively high concentrations of carbonate and magnesium might be caries susceptible.[25]

5) Fluoride

Fluoride concentration in the enamel falls exponentially from the surface inwards towards a plateau in the middle third of the enamel.[17, 26] Fluoride has been shown to substitute hydroxide ions in the apatite crystals forming fluorapatite. The fluoride-containing apatites have high stability and crystallinity and therefore more resistant to acid attack. The presence of fluoride also enhances the growth and size of the apatite crystals, providing a reduced surface area for acid attack.[13]

5) Zinc

The distribution of zinc is higher on the outer than the inner enamel surface.[27] It has been shown that the zinc concentration of teeth vary in different geographic areas in USA.[28] However, little is known of the significance of zinc in teeth. The source of zinc may be due to contamination from dental fillings in the tooth or neighboring teeth.[29, 30]

6) Lead

The concentration of lead in human teeth increases with age up to early adulthood and then remains fairly constant.[29] Lead level was found to be highest on enamel surface and dentino-enamel junction (DEJ).[31] Lead found in human enamel is likely to be a result of ingestion from the polluted urban environment.[32]

7) Silicon

The effect and distribution of silicon has not been studied extensively. It is believed silicon in teeth may be a result of contamination from dental fillings in teeth.[29] In a recent study, silicon has been incorporated into toothpaste as SiF, which was shown to increase the acid resistance of enamel and therefore could be considered as a future potential anti-cariogenic agent.[33]

These minor inorganic minerals and trace elements when present may be incorporated into the enamel and affect the chemical composition of enamel. Therefore, the chemical environment of the saliva in the oral cavity can have an affect on the developing tooth and teeth that develop at approximately the same and expose to the same chemical environment may have similar chemical composition. Using surface scan methods, the chemical make-up of the enamel surface may be elucidated.

1.2.2 SURFACE SCAN METHODS

Previous studies on the chemical composition of human enamel have been based primarily on the whole thickness of enamel samples.[34, 35, 36] After sectioning of the enamel from the dentin, the enamel was grounded to powder and through analytical chemistry, the composition of the enamel determined. This chemical composition represented the average of the entire thickness of the enamel and was not a true reflection of the surface layer of enamel where orthodontic bonding took place.[26] With advances in technology, the characterization of enamel can be restricted to its outer surface layer. A review of two of these surface science techniques, X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) is presented.

1.2.2.1 X-RAY PHOTOELECTRON SPECTROSCOPY (XPS)

XPS is a non-destructive method of determining the chemical composition of a surface. XPS can detect all elements with the exception of hydrogen and helium.[37] A beam of monochromatic or polychromatic X-ray photon (hv) is directed onto the material surface to excite several core energy levels of the electrons of the atoms that exist within the top layer of the material. Electrons from all the orbitals of the atoms with a binding energy (E_b) less than the X-ray energy are excited but not with an equal probability. XPS spectra are obtained by measuring the number of electrons and the kinetic energy (KE) of the electrons that have escaped from surface of the material being analyzed. The photoelectrons that are able to escape are those at the top 1 to 10 nm of the material while many of the excited electrons in the deeper layer do not have sufficient kinetic energy to escape and are either recaptured or trapped in various excited states within the material.[38] The kinetic energy of the escaping photoelectrons therefore limits the depth from which excited electrons can escape thus giving XPS its high surface sensitivity and a sampling depth of a few nanometers.

Since energy is conserved and the energy of the X-ray photon source is known, the electron binding energy (E_b) of each of the emitted photoelectrons can be determined using the equation:

$$E_b = E_{hv} - E_{ke} - \Phi$$

where E_b is the binding energy of the electron emitted from a particular electron configuration of an atom, E_{hv} is the energy of the X-ray photons used to excite the material, E_{ke} is the kinetic energy of the emitted electrons measured by the instrument and Φ is the work function of the spectrometer. The measured binding energy is characteristic of each element but can be slightly altered by the chemical state of the ejected photoelectrons. Hence XPS can provide chemical bonding information.

XPS is performed under ultra-high vacuum (UHV) condition. When the pressure is lowered, the mean free path, or the average distance the particle travels betweens collisions with other particles, is increased allowing the ejected photoelectrons to be counted by the detector instead of being scattered away and lost from analysis due to collisions with other photoelectrons or gas molecules. An HUV environment also has the benefit of allowing an undisturbed primary X-ray emission and reducing surface contamination from the atmospheric environment.[37]

In the dental literature, XPS has been utilized to investigate the chemical interaction of syntehsized polyaklenoic acid with enamel and synthetic hydroxyapatite,[39]to study the mechanism of acid etching of polyacrylic acid[40] and maleic acid [41]on enamel surface; to monitor the adsorption of active agents from six mouthrinses,[42] to study the adsorption of salivary constituents on enamel,[43] and to examine several dental biomaterial surfaces.[44, 45, 46, 47, 48, 49, 50] Yoshioka *et al.* [51] studied the adhesion/decalcification mechanisms of acid interactions of five carboxylic acids (citric, lactic, maleic, and oxalic) and two inorganic acids (hydrochloric and nitric) with enamel and two synthetic hydroxyapatite (HAp) powders using XPS. No study was found that used XPS to analyze the chemical composition of human enamel surface and its relation to orthodontic bonding strength.

However, many studies have utilized XPS to investigate the enamel surface for a variety of other reasons. Alan *et al.*[52] was the first research group to determine the chemical composition of the labial surface of a human enamel using XPS. The chemical composition was found to be consistent with the surface being predominantly being calcium hydroxyapatite with trace contaminants of Na, Si, N and S. Ziglo[53] used XPS to ascertain if the demineralization resistance of human enamel imparted by argon laser was due to changes in carbonate level. Through the high resolution scan of C 1s peak which contains all the carbon-bond information, the atomic concentration percentage of carbonate was determined. The results showed that carbonate content of enamel after argon laser irradiation was not significantly different than the control group, suggesting that any demineralization resistance was not due to alterations in the carbonate content.

The high surface sensitivity and the ability to provide chemical shift information about the chemical species present on the surface of a material being analyzed make XPS a valuable and unique surface analysis tool in the study of human enamel.[38]

1.2.2.2 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning electron microscopy (SEM) has traditionally been the method used to study the qualitative etching pattern of enamel[9, 54, 55, 56, 57, 58] and has continued to be the technique of choice for such purpose[59, 60, 61, 62] due to its striking ability to provide a high depth-of-field topographic image.[63] The basics of SEM is as follows. A beam of electron is generated in a vacuum and accelerated to an energy in the range of 1-40 keV. The electron beam is then collimated by a series of electromagnetic condenser lenses, focused by objective lenses, and scanned across the specimen surface through electromagnetic deflection coils. The interaction of the electron beam with the specimen causes the generation of many signals. The most commonly used signals in producing the conventional SEM image are the secondary electrons which are emitted specimen electrons at the uppermost few nanometers of the specimen.[63] These secondary electrons are detected by a scintillation material that produces flashes of light from the electrons. The light flashes are converted into an amplified electrical signal by a photomultiper tube. By correlating the sample scan position with the resulting signal, an image can be formed and displayed on a cathode ray tube. Since the amount of electron scattering depends on the angle of the specimen surface relative to the incident electron beam, SEM image has highlights and shadows that give it a 3-D appearance.

The samples to be examined by SEM must be able to withstand the electric currents produced by the bombardment of the electron beam. Insulator specimens such as enamel that do not readily conduct electricity must first be coated with a thin layer of conductive material to avoid damage caused by charges that can build up in the sample. This coating process is accomplished using a sputter. A sputter coater will deposit a nanometer-thick layer of conductive material on the sample surface while retaining the original contour.

In orthodontic bonding literature, SEM has been used to establish the classification of the qualitative enamel acid etching pattern,[9, 54, 55, 56, 57, 64], to study the influence of acid concentration on the enamel etching,[55, 59, 65, 66, 67] to study the duration of acid application on the quality of the etch [57, 68, 69, 70, 71] and to study the effect of different type of acids on the enamel etch.[62, 69, 72, 73, 74, 75] These studies will be explored in detail in the bonding and etching pattern sections of the literature review.

It is clear from the literature review that SEM has been a widely used method for studying the qualitatively etching pattern after the application of acid prior to orthodontic bonding. No study however, was found that examine the chemical composition of enamel as a factor in explaining the resulting qualitative ~~acid~~ etch pattern.

1.2.3 ORTHODONTIC BONDING

In modern orthodontics, the ability to bond orthodontic brackets to enamel is fundamental to the routine practice of orthodontic treatment. This is achieved by etching the enamel surface with an acid, usually a 37% w/w phosphoric acid. A composite resin is then flowed into the etched enamel surface to create resin tags which act as mechanical retention. The success or failure of fixed orthodontics depends to a large extent on achieving a durable bond to the enamel.

Acid etching of enamel to allow for adhesive bonding is however, not a new concept. In 1955, Buonocore [76] introduced the technique of acid etching of enamel. Since then, it has undergone extensive research and changes. Acids of different types, concentrations, and duration of applications have been studied on enamel and dentin, for restorative as well as orthodontics purposes.

For bonding to be considered successful in orthodontics, the bonded brackets must have adequate bond strength to prevent de-bonding prior to the completion of treatment, with various studies suggesting a range from 2.8 MPa to 10 MPa as being adequate for clinical situations.[3, 77] This bond strength must also be achieved consistently and it must not be too strong to cause fracture of enamel when brackets are removed at the end of treatment.

1.2.4 FACTORS CAUSING ORTHODONTIC BOND FAILURE

The unplanned debonding of brackets prior to completion of treatment is a major drawback of fixed orthodontics. The bond failure rate ranges from 0.5% to 16% [6, 7], in various orthodontic practices. Numerous factors have been proposed and these include:

1. Operator technique

There are a number of factors which are dependent upon operators. Grubisa *et al.* [78] found significant differences between operators with the use of self-etching primer. Clinicians who were already using self-etching primer tend to drift away from manufacturer's instructions and introduce technique modification while clinicians who were first time user of self-etching primer followed the instructions. This invariability introduced inter-operator variability. Finger *et al.* [79] compared the quality of margin restoration placed by five practicing general dentists using a new one-bottle adhesive system having been provided only with the manufacturer's instruction. The authors noted that not all operators read the instructions fully which resulted in different restoration margin quality. Inherent operator differences in technique may have an effect on bond failure rate.

2. Prophylaxis

In the mouth, saliva will deposit proteins quickly over a tooth surface that has been cleaned. This deposit reduced the surface reactivity of enamel [80, 81] and therefore it has become an accepted practice to perform a prophylaxis of tooth surface prior to etching. However, Barry [82] and Lindaeur *et al.*[83] found no significant difference in orthodontic bond failure whether prophylaxis was used. The authors concluded that prophylaxis is not essential in achieving satisfactory bond strength.

3. Etchant

The type of etchant used and its duration of application;[70, 84, 85] the duration of water rinse for removal of acid and drying of etched surface prior to adhesive application.[86, 87] are all variables that can affect bond failure rate.

The acid etchant used clinically is available as either a liquid or gel. Walker and Vann[88] found liquid acid to be able to produce a more even etch pattern than acid gel. However, the depth of resin tag penetration and bond strength were not significantly different.

Brannstrom *et al.*[56] found no difference in surface irregularity when comparing gel and liquid acid etchants, which agreed with the study done by Guba .[70]. Acid gel, has the clinical advantage in that it is easier to place in a controlled and confined area.

4. Adhesive and bracket system

The type of adhesive and bracket system used can affect the bond strength. Fox *et al.*[89]compared the *in vitro* bond strength of a conventional composite resin to a fluoride releasing composite and a glass ionomer cement and found that conventional composite had the highest mean strength. Bishara *et al.* [90]investigated the bond strength between precoated and uncoated ceramic and metal brackets and found that precoating the brackets has minimal effect on ceramic brackets while the bond strength of precoated metal brackets was significantly reduced when compared to the uncoated metal bracket. Mandall *et al.*[91] performed a systematic review to evaluate which orthodontic adhesive has the lowest bonding failure and was effective at preventing enamel decalcification. The authors could not draw any conclusions from the systematic review as many of the studies had poor quality of reporting of the results. Suggestions were made to improve the quality of reporting clinical trials in future orthodontic adhesive study.

5. Moisture and saliva contamination

The clinician's manual dexterity and ability to keep moisture from contaminating the etched surface has been shown to reduce clinical bond failures on posterior teeth. [90, 92]

6. Masticatory forces

There is a great variation between patients in their masticatory forces. [4] Excessive chewing forces may result in higher bond failure. [6, 93] It has been suggested that brackets bonded to mandibular teeth are more prone to failure because of greater masticatory forces being exerted on these brackets but this has been refuted by Carstensen. [84]

7. Degradation of the adhesive

Matasa [94] suggested that *Pseudomonas aeruginosa* and *Streptococcus mutans* which are oral bacteria found in the mouth can degrade dental composite resins, corrode stainless steel brackets and cause bond failure.

8. Effects of tooth type

The outer surface of enamel is the area to which an etchant is applied prior to orthodontic bonding. Variation in the surface enamel may therefore affect the results of etching and bond failure. Study by Sheykholeslam *et al.* [95] has shown that prismless or aprismatic enamel, where no prisms reached the outer enamel surface was more resistant to etching and negatively affected the bonding. Using scanning electron microscopy, Whittaker [96] determined that the thickness and distribution of prismless enamel varied between different tooth types, tooth surfaces and between deciduous and permanent teeth. Taken as a whole, deciduous teeth were more likely to have an aprismatic surface zone than permanent teeth. This aprismatic zone, when present, occupied a larger zone in anterior than posterior teeth. The implications of these finding may be important in relation to the results of etching and bonding. The finding suggested that enamel bond strength study use the same tooth type in order to achieve meaningful comparisons. This finding is in agreement with the result of the clinical studies by two other research groups[4, 97] where the bond failure rate was found to be highly dependent on the tooth position.

1.2.5 ORTHODONTIC BONDING RESIN STUDY DESIGN

There are numerous bonding systems available in orthodontics. The literature contains an exceedingly large number of studies both *in vitro* and in a clinical setting. It is however, difficult and often impossible to compare the data directly between these studies due to the large number of materials and methods used.

Fox *et al.* [98] carried out a literature review on *in vitro* bond strength testing in orthodontics and recommended the following protocol in order to standardize future bonding study:

1. Premolar surface enamel extracted from adolescent orthodontic patients should be used for bonding study.
2. Teeth should be stored in distilled in water prior to bonding
3. After bonding, but prior to the study, teeth should be immersed in water at 37°C for 24 hours.
4. Debonding should take place on an Instron® or equivalent machine at a cross-head speed of 1mm per minute.
5. The point of application and direction of the debonding force should be the same for all specimens.

6. At least 20 and preferably 30 specimens should be used for each study.
7. Bond strengths should be quoted in either Newtons or Megapascals.

The above guidelines were used in the present study except the teeth were stored in 0.1% thymol after extraction and thermocycled 750 times between water baths of 5°C and 55°C prior to debonding. These modifications were made to follow the guidelines set up by previous University of Alberta Orthodontic Graduate residents.

For clinical orthodontic bonding study, Mandall *et al.*[91] after performing a systematic review to assess the clinical orthodontic adhesive bond failure rate and decalcification around brackets suggested the following guidelines for in vivo orthodontic bonding study:

1. The study should be carried out as a prospective randomized clinical trial.
2. Bond failure should be followed to the completion of patients' fixed appliance treatment.
3. Sample size should be calculated before the study.
4. Statistician should be consulted for appropriate statistical analysis and study design.
5. The study must have a clear inclusion and exclusion criteria.
6. Patient drop out and withdrawal should be stated and statistical analysis modified if appropriate
7. Occlusal interference that may affect bond failure should be noted.
8. The study should be double blind.

9. The standard deviation of the bond failure should be recorded

10. Measuring decalcification as a secondary outcome where appropriate.

1.2.6 ACID ETCHING

1.2.6.1 PRINCIPLES OF ACID ETCHING

Without etching, enamel is a poor substrate for bonding as it is porous, not smooth and has low surface reactivity.[80, 86] Further, inside the mouth, salivary proteins, called pellicle, quickly covers a clean tooth surface and reduces the surface reactivity of the enamel.[80, 81] Treating the enamel surface with acid will remove the pellicle layer, some of the mineralized component of enamel, expose the enamel prisms and increase the surface energy and wettability of the enamel.[99] This leaves a highly reactive surface with increased porosity and surface areas for which the adhesive resin can flow and penetrate into to create resin tags for a mechanical bond to form.

The depth of the etch that is achieved and the amount of enamel that is removed is dependent on the nature of the enamel itself. Aprismatic enamel which is found on primary dentition enamel and in greater thickness in the posterior than anterior permanent dentition enamel may limit the extent of acid etching and reduce the resulting bond strength.[96, 100] Further, the depth of enamel etch is dependant upon the degree of calcification and the fluoride content of the surface enamel. As the surface fluoride content increases, the resistance to acid etch also increases.[101, 102, 103] Lee *et al.* [104] found an increase in failure rate of sealants in patients who live in areas where water fluoride level was higher. The fluoride content of surface enamel has been shown to be a function of systemic intake during tooth development, which includes topical application of fluoride from toothpaste, mouth rinses, varnishes, gels, drinking water, and food and drinks.[105]

1.2.6.2 EFFECTS OF VARIOUS ACIDS

Since the pioneering work of enamel etching with 85% phosphoric acid by Buonocore[76] numerous acids have been proposed for enamel etching which included citric, oxalic, maleic and nitric acid. Phosphoric acid, however, remained the gold standard against which all other acids have been evaluated.

Retief[106] compared the qualitative etching effect of citric acid to phosphoric acid using SEM. Citric acid was found to be slower in dissolving enamel and has the mildest effect on the enamel resulting in a smoother topographical surface than phosphoric acid. Pyruvic acid as an etching agent was studied by numerous authors who found it capable of producing adequate bond strength and similar surface etching patterns to phosphoric acid but in less time.[74, 107, 108] The clinical use of pyruvic acid however, was limited because it was unstable and prone to degradation.[109]

Nitric and maleic acids have also been evaluated and are commercially available for restorative purpose. The acid etch pattern of 2.5% w/w nitric acid on extracted premolars at various application times has been examined with scanning electron microscopy.[69]. It was found that by increasing the duration of nitric acid application to enamel surface, there was a significant increase in the amount of better quality etch. However, when compared to 37% phosphoric acid, nitric acid was less effective at producing a good-quality etch for all application times. Blosser[110] also found that the nitric acid was capable of producing optimal surface topography as the etch time increased, but the resultant bond strength was found to be weak. Maleic acid at 10% w/w found in Scotchbond Multi-purpose system (3M Company, Monrovia, California, USA) has shown to be incapable of producing similar bond strength to enamel when compared to phosphoric acid.[75, 111] The mechanism of maleic acid has recently been shown to be the result of its ability to simultaneously decalcify and chemically adhere to hydroxyapatite on the enamel surface.[112]

Despite the research into alternative acids for enamel etching, the gold standard for enamel etching is phosphoric acid. At a concentration of 30-40% w/w, phosphoric acid is the most widely used orthodontic etchant.[69]

1.2.6.3 EFFECTS OF ACID CONCENTRATION

When acid is applied to enamel, a characteristic etching pattern is produced as a result of dissolution of enamel. The etch pattern is a reflection of the solubility of the enamel due to its morphological and chemical variation. The depth of etch and the amount of enamel removal is dependent on the acid concentration and the chemical composition of the enamel.[67, 113, 114]

While Buonocore first used phosphoric acid at a concentration of 85%,[76] the concentration in clinical use today has progressively decreased to a range of 30-40%.

This is a reflection of studies[80, 113] showing that weaker phosphoric acid concentrations is able to produce more microscopic pores and a higher bond strength than stronger concentrations, as acids at high concentration simply remove the enamel substrate with few microscopic pores produced.

Carstensen[84] studied the effects of various phosphoric acid concentrations from 2 to 40% w/w on surface enamel. There were large variations in the etching patterns produced with the lower concentrations producing a poorer definition of enamel prisms. But in a clinical study by Sadowsky *et al.*[115] no significant bond failure rate was observed when the phosphoric acid concentrations was varied between 15 and 37%. This would suggest that factors other than acid concentration may have more effect on the clinical bond failure rate.

1.2.6.4 EFFECTS OF ACID ETCHING DURATION

In the clinical setting, the most commonly used etched time is between 15 and 60 seconds.[115, 116] An in vitro study by Malferrari *et al.*[117] has shown that the most prominent etching pattern was produced when a longer etching time was used. However, no significant difference in bond strength was observed. This is in agreement with the study by Olsen *et al.*[118] where no difference in bond strength was found when 10, 15, 20 or 30 seconds etching time was used. Sadowsky *et al.*[115] and Kinch *et a*l.[4] also found that there was no significant difference in the bond failure rate whether 15 second or 60 second etching time was used. Clinically, there appears to be no advantage in using a long acid etching time.

In clinical practice, most clinicians use an acid etch time of 30 seconds and a phosphoric acid concentration of 30-40% w/w.[116]

1.2.6.5 EFFECTS OF TOOTH TYPE

The orthodontic bond strength of a bracket is dependent on the type of tooth being investigated (premolar, molar). Hobson *et al.*[119] found that the shear bond strength of stainless steel bracket was significantly different and dependent on the location and the type of tooth. The highest mean shear bond strength was on the mandibular molar teeth, while the lowest was found on maxillary first molars. Maxillary anterior teeth were found to have greater bond strength than maxillary posterior teeth, while the reverse was true in the mandibular dentition. Bond strength was noted to be statistically different between the maxillary and mandibular second premolars, while the maxillary and mandibular first premolar bond strength were similar.[119] This finding is in agreement with the result of the clinical study by Kinch *et al.*[4] where the bond failure rate was found to be highly dependent on the tooth position.

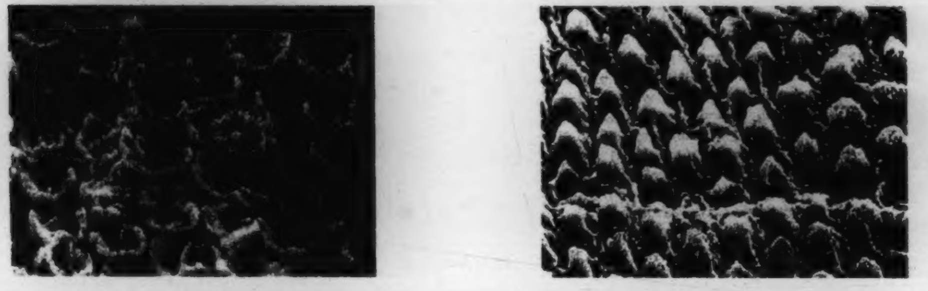
The finding suggested that enamel bond strength study use the same tooth type in order to achieve meaningful comparisons.

1.2.7 ETCHING PATTERNS

After phosphoric acid is applied to enamel surface, an etch pattern is produced. The surface enamel etch patterns have been studied by a number of researchers using scanning electron microscopy (SEM).[9, 54, 55, 56, 57, 120] Silverstone *et al.* [54] were the first to describe the etching patterns as seen under SEM. They classified the patterns into three types: type 1 etching pattern in which prism core material was preferentially removed leaving the prism peripheries intact producing a characteristic honeycomb appearance; type 2 etching pattern in which the peripheral regions of prisms were removed preferentially leaving prism cores remaining relatively unaffected producing a cobblestone appearance; type 3 etching pattern in which random patterns were observed with some areas corresponding to type 1 and 2 patterns and areas in which the etching patterns could not be related prism morphology. The authors reported type 1 honeycomb appearance was the most commonly observed etching pattern and suggested the basis of various etching patterns was due to chemical composition and crystallite orientation. Brannstrom *et al.* [56] and Nordenvall *et al.* [57] proposed a sliding 3 point scale of surface irregularity from 0 to 3; 0 represented a smooth surface, and 3 represented optimum irregularities. Galil and Wright [55] described five distinct etching patterns and their classification has become widely accepted and a number of studies have used their classification.[116, 121, 122, 123, 124] Type 1 and 2 paralleled the findings of Silverstone *et al.*[54]; type 3 was a mixture of type 1 and type 2 patterns; type 4 represented pitted enamel surfaces; type 5 appeared as flat and smooth surfaces. The authors found that type 1, 2 and 3 were mainly observed in the coronal and middle thirds of the buccal surfaces while type 4 and 5 patterns were mainly located in the cervical third of the buccal surfaces. Hobson *et al.*[120] modified the etching pattern proposed by Galil and Wright by combining Type 1 and Type 2 into a single category as both represented the ideal etch pattern. Hobson's etching pattern classification is as follows.[120]

Type 1 represented well-developed conventional etch pattern with well-defined prisms

Fig 1.2: From Journal of Pediatric Dentistry, 1979; 232; SEM photomicrograph x 1500

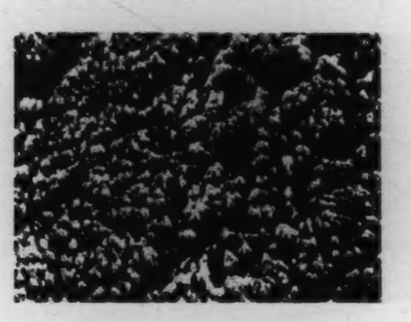


Type 2 has discernible prisms but prisms are poorly defined

Fig 1.3: From Journal of Pediatric Dentistry, 1979; 232; SEM photomicrograph x 1500

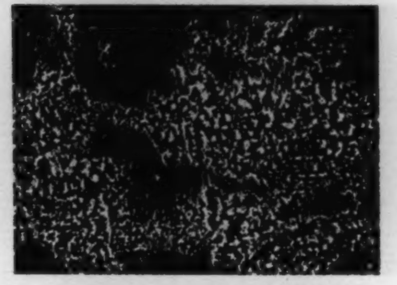
Type 3 has no prism definition but surface roughening has occurred

Fig 1.4: From Journal of Pediatric Dentistry, 1979; 232; SEM photomicrograph x 1500



Type 4 represented flat smooth surface

Fig 1.5: From Journal of Pediatric Dentistry, 1979; 232; SEM photomicrograph x 1500



Hobson's enamel etching classification was used in this study.

1.2.8 SUMMARY

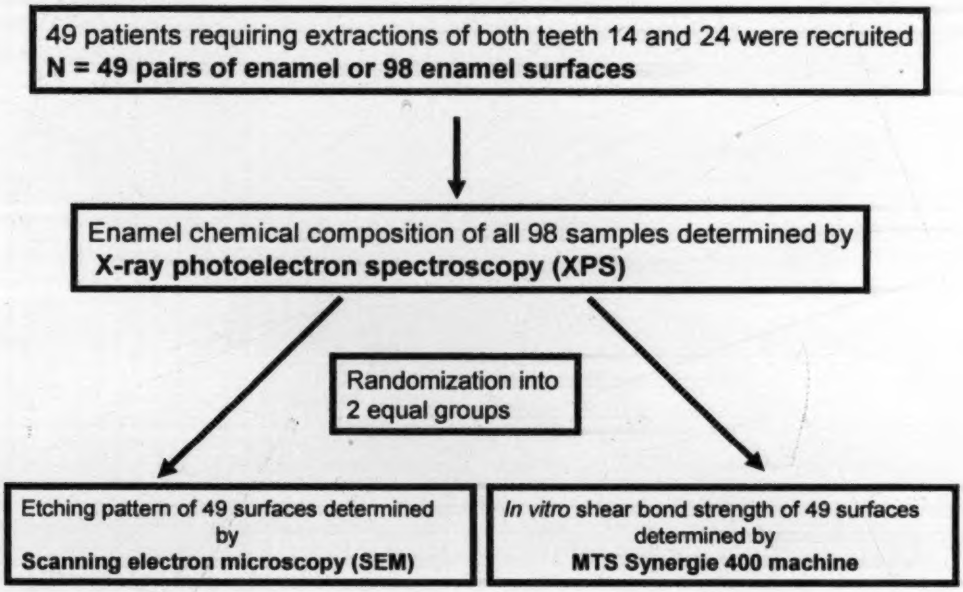
Since Buonocore's initial research[76] a great deal of research has been undertaken to understand the factors that affect orthodontic bonding strength. Much is now known about the effects of different types of acids, the duration of acid application and different concentration of acids on the bracket bond strength both *in vivo* and *in vitro*. There has been however, scant information and research on the effect of the chemical composition of the enamel and its effect on the etching pattern and subsequent bond strength.

The use of XPS can provide the surface chemical composition of enamel while SEM can provide information on the qualitative acid etching pattern. By relating the chemical composition of the enamel surface to its qualitative enamel acid etching pattern and the resulting bond strength, the inter-relationship between these variables can be determined.

1.3 STATEMENT OF PROBLEM

Many causes of orthodontic bond failure have been studied extensively. The surface of human enamel to which the etchant is applied to create microscopic pores for mechanical bonding of orthodontic brackets, however, has not been the subject of systematic study. The first part of the study will investigate the surface chemical composition of human enamel and determine if it can be a predictor of *in vitro* shear bond strength. The second part of the study will investigate whether the chemical composition of human surface enamel can be a predictor of the qualitative etching pattern of enamel surface. The last section of the study will examine the inter-relationship between the quality of etching pattern and *in vitro* shear bond strength.

Figure 1.6 Flow chart of the methodology of the current research


49 patients requiring extractions of both teeth 14 and 24 were recruited
N = 49 pairs of enamel or 98 enamel surfaces
Enamel chemical composition of all 98 samples determined by
X-ray photoelectron spectroscopy (XPS)
Randomization into 2 equal groups
Etching pattern of 49 surfaces determined by
Scanning electron microscopy (SEM)
In vitro shear bond strength of 49 surfaces determined by
MTS Synergie 400 machine

1.4 SIGNIFICANCE OF THE STUDY

The success of fixed orthodontic treatment depends to a large extent on the ability to achieve a strong and durable bond to the enamel surface. Each patient seeking orthodontic treatment can be viewed as not only presenting a unique dental problem but a unique surface characteristic on their enamel as well. If a relationship does indeed exist between the surface characteristics and bond strength, future study on bonding can focus on developing a simple and rapid diagnostic test to characterize the enamel surface and manufactures can custom made adhesives unique to each patient. It would be conceivable that when the future children of these participants are ready for treatment with braces, the orthodontists of the future can predict in advance the difficulty of the treatment with respects to possible bond failure rate and inform the patients in advance and plan the treatment accordingly.

1.5 RESEARCH QUESTIONS

1.5.1 PRIMARY RESEARCH QUESTIONS

1. To determine if the surface chemical composition of extracted maxillary first premolar enamel can be used as a predictor for bond strength in the *in vitro* setting.

2. To determine if the surface chemical composition of extracted maxillary first premolar enamel can be used as a predictor of qualitative etching pattern.

1.5.2 NULL HYPOTHESES FOR PRIMARY RESEARCH QUESTIONS

1. The surface chemical compositions of extracted maxillary first premolar enamel cannot be used as a predictor of bond strength in the *in vitro* setting.

2. The surface chemical compositions of extracted maxillary first premolar enamel cannot be used as a predictor of etching pattern qualitatively.

1.5.3 SECONDARY RESERARCH QUESTIONS

1. To determine if there is a difference between the surface chemical composition of right and left maxillary first premolar enamel.

2. To determine if the surface etching morphology of extracted maxillary first premolar enamel can be used as a predictor for bond strength in the *in vitro* setting.

1.5.4 NULL HYPOTHESES FOR SECONDARY RESEARCH QUESTRIONS

1. There is no difference between the surface chemical composition of right and left maxillary first premolar enamel.

2. The surface etching morphology of extracted maxillary first premolar enamel cannot be used as a predictor of bond strength in the *in vitro* setting.

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Chapter 2

Chemical Composition of Maxillary Right and Left First Premolar

2.1 INTRODUCTION

The study of the surface chemistry of enamel that began in the 1950 had a focus on its relationship to caries susceptibility. From approximately 1950 to 1970, studies on the chemical composition of the human enamel were carried out using analytical chemistry, which destroyed the samples after analysis therefore, further experimental work on the samples were not possible.[1, 2, 3, 4, 5, 6] In addition, these studies were performed with small sample size and utilized teeth of different types. Due to the heterogeneous nature of enamel surface, the results from studies using different tooth types cannot be directly compared to each other.[7] From 1980's and onwards, with the acceptance of cosmetic bonding, the attention was shifted from caries research to bonding of dental material to tooth structure. There was, however a lack of study directly relating chemical composition of enamel to bond strength.[8] Most studies, instead, focused on the dental material itself instead of tooth chemical composition.[9, 10] In the present study, a large sample size consisting of only one tooth type - maxillary first premolar was used. The chemical composition of the outermost nanometer of these enamel surfaces was systematically examined using x-ray photoelectron spectroscopy (XPS). The chemical composition of maxillary right first premolar was also compared to the left maxillary first premolar. A lack of surface chemical difference between the right and left sides will allow future studies that relate the chemical composition to bond strength and enamel etching pattern. With chemical composition as a common denominator, and by separating the teeth into a scanning electron microscopy (SEM) etching pattern group and a bond strength group, the inter-relationship between chemical composition, etching pattern and bond strength can be determined. This knowledge of the chemical composition of enamel surface will begin to provide a rational basis for the wide variation in bond strength frequently noted in laboratory and clinical settings in future study.

2.2 MATERIALS AND METHODS

2.2.1 SAMPLE SELECTION AND HANDLING

After approval from the Health Research Ethics Board of the University of Alberta (Appendix A) and written consent from patients for use of their teeth in the study (Appendix B) the recruitment process (Appendix C) for extraction of maxillary first premolars was initiated. The reason for choosing premolar teeth for the present study was based on the fact that these teeth are the most likely teeth to be extracted during orthodontic treatment. The buccal surface was used because this is the surface where orthodontic brackets are placed.

The calculated sample size required 58 patients and was based on using five chemical elements to explain 20% of the variation seen in orthodontic bond strength at a power of 80% with an α of 5% (Appendix D). With the time and cost restrictions of using the surface analysis equipment, a total of 51 patients requiring both maxillary right and left first premolar extractions as part of orthodontic treatment at the University of Alberta Orthodontic Graduate Program were recruited for the study. However, 2 patients were excluded as their enamel samples fractured during the transport process to the engineering building for XPS analysis. Therefore, a total of 49 patients were included in the study. The selection criteria for the sample required that the teeth to be without enamel defect or developmental disturbances and teeth that were not bonded previously. There was no age restriction for this study.

The teeth used in the study were extracted by a single board-certified periodontist. Teeth with cracks due to pressure of the extraction forceps were not included. After extraction, the teeth were gently wiped clean with gauze to remove cellular debris and each stored separately in a jar containing 0.1% thymol solution. The jar was labeled with a code and the principal investigators were blinded with regards to the identity of the teeth. For organizational purpose, the extracted teeth were stored in 0.1% thymol for 14 days. At day 14, the teeth were rinsed with distilled water prior to sectioning. The buccal portion of the enamel was sliced longitudinally in a mesio-distal direction at the line angles from the remaining tooth using a diamond disc (Brasseler Dental Instrumentation, Savannah, Georgia, USA). Each of the sectioned enamel samples was rinsed with copious distilled water prior to being stored in individual compartments of a pill organizer. When 8 enamel samples have been obtained, the samples were sent to Alberta Center for Surface Engineering and Science (ACSES) at the University of Alberta for surface analysis using X-ray photoelectron spectroscopy (XPS).

2.2.2 SURFACE TESTING WITH XPS

The composition near the buccal surface of the enamel is likely to be different from the bulk of the enamel. It is therefore important to use techniques that test only the surface of the sample. XPS is a surface sensitive tool that is able to provide the chemical composition of the outer nanometer layer of the buccal portion of the sectioned enamel.

The theory of XPS has been described in the literature review section of the thesis. A brief summary reviewing its theory is presented here. XPS is a method used to determine the chemical composition of a surface. The analysis is done by irradiating a sample with x-rays to produce photoelectrons from the surface layers of atoms in a solid sample. The kinetic energy of the escaping photoelectrons limits the depth from which it can emerge, giving XPS its high surface sensitivity and sampling depth of a few nanometers.[11] The emitted photoelectrons are collected and analyzed by an instrument to produce a spectrum of emission intensity versus electron binding energy. As each element has a unique set of binding energies, XPS has the capability of identifying the different elements on the surface of a sample. In addition, the concentration of the elements can be quantified. Small shifts in the binding energies can provide information about the chemical states of the surface atoms. An advantage of XPS is its relatively non-destructive technique compared to other methods of surface analysis. No surface species are removed during XPS analysis, and the soft X-ray source used for excitation avoids the many problems associated with thermal degradation of sensitive materials.[12]

The XPS scans in this study were acquired using an AXIS ULTRA XPS (Kratos Analytical, Manchester, UK, Fig 2.1), which utilized monochromatic AlKα x-rays at hν = 1486.6 eV. The x-ray gun was operated at 210 W. The sample was placed relative to the analyzer to give a 90 degree takeoff angle, where the takeoff angle is defined as the angle between the surface normal and the axis of the analyzer lens. All samples had a survey spectrum (0-1100 eV), as well as high resolution spectra of the elements fluorine, nitrogen, oxygen, calcium, carbon and phosphorous performed. The survey scan was performed with a pass energy of 160eV, while 20 eV was used for the high resolution spectra. The high resolution scan is conducted at a step of 0.3 eV and 0.1 eV for the survey and high resolution scans respectively.

Figure 2.1 AXIS ULTRA XPS (University of Alberta, Surface Sciences)

The samples were loaded via an entry lock and degassed under high vacuum (298K and 10^{-10} torr) until the pressure was down to approximately 1 x 10^{-6} torr. Then the samples were transferred into the analytical chamber with base pressure of 3-4 x 10^{-10} torr and working pressure of about 2-5 x 10^{-8} torr. The samples were exposed to AlKα at a beam size of 1200μm x 80μm for alignment and signal optimization prior to analysis. The size of the area analyzed was 700μm x 300μm. The total signal accumulation time per specimen consisted of 120 to 363 seconds. For insulating samples, the coaxial charge neutralizer filament was used, and the binding energy scales for the samples were referenced by setting the C 1s band of adventitious carbon to 258.0 eV.

Data acquisition and analysis was performed by Kratos XPS Casa software on a Sun Computer System. The analysis of the spectra was performed via:

- Peak shape and background method: Following a Shirley-type background subtraction, the individual photoemission features are fitted with representative Gaussian distributions using least-squares optimization. The peak positions, amplitudes, and full width at half maximum parameters are obtained from the Gaussian distribution analysis. The peak areas correspond to the area with respect to the background subtraction.
- Quantization method: The atomic concentrations are calculated using the algorithm and sensitivity factors contained in Kratos Analytical Software.

Survey scans and high resolution scans of the C 1s, Ca 2p, O1s, N 1s, P 2p and F 1s were recorded. The high resolution spectra were labeled with the abbreviated name, followed by the quantum number of the ejected photoelectrons. The C 1s spectrum was used to analyze the percentage of carbon present as carbonate. Four carbon states were fitted to the C 1S peak. The components were C-C / C-H at 285 eV. The next carbon species corresponded to ether carbon C=O, followed by CO_2^- and carbonate carbon CO_3^- which has the highest binding energy in the spectrum.

2.3 XPS RESULTS

The use of XPS detected a total of 12 elements on the buccal surface of maxillary first premolars. Not all the samples contained the 12 elements, and only a few samples had chlorine and magnesium detected. These elements were sodium (Na), zinc (Zn), oxygen (O), nitrogen (N), calcium (Ca), carbon (C), phosphorus (P), silicon (Si), chlorine (Cl), sulphur (S), fluoride (F) and magnesium (Mg). The main elements were Ca, P, O, C and N while other elements were in minor amount. There was a tendency for each of the detected elements to exhibit a large range of values. Carbon was further analyzed into its components to detect the percentage of carbonate (CO_3) to facilitate calculation of CO_3 / P ratio which is believed to be an indicator of enamel's susceptibility to acid attack.[13] An example of a XPS survey scan and a high resolution scan of carbon depicting the elements detected in the right and left maxillary first premolar from the same subject are illustrated in Figures 2.2 and 2.3. The descriptive statistics of the atomic concentration percentage of the 12 elements, CO_3^- and CO_3^- / P ratio is shown in Table 2.1 and the raw data displayed in Appendix E.

Table 2.1 Descriptive statistics for the detected elements, measured in % atomic concentration, with n = 98

	Minimum	Maximum	Mean	SD	Coefficient of Variation
Na	0.06	1.89	0.39	0.26	0.67
Zn	0.00	0.58	0.14	0.12	0.86
O	22.26	44.38	31.37	4.82	0.15
N	2.82	10.76	6.76	1.85	0.27
Ca	1.37	11.26	5.58	2.27	0.40
C	30.66	63.16	50.66	7.28	0.14
P	1.17	8.27	3.95	1.56	0.39
Si	0.00	2.10	0.60	0.31	0.52
Cl	0.00	2.16	0.04	0.26	6.5
S	0.00	1.11	0.24	0.23	0.96
F	0.00	0.59	0.19	0.18	0.95
Mg	0.00	0.39	0.02	0.08	4
CO_3	0.00	11.11	3.01	1.70	0.56
CO_3/P	0.00	5.24	0.85	0.80	0.94

Figure 2.2 An illustrative example of superimpositions of survey scans revealing no difference in any of the detected elements between right and left maxillary first premolars

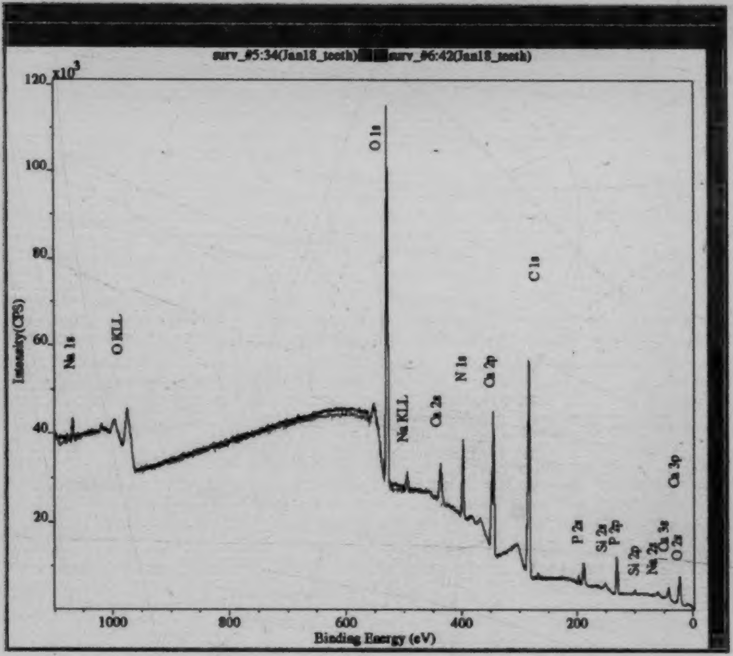


Figure 2.3 An illustrative example of superimpositions of high resolution scans revealing no difference in the sub-components of carbon between right and left maxillary first premolars

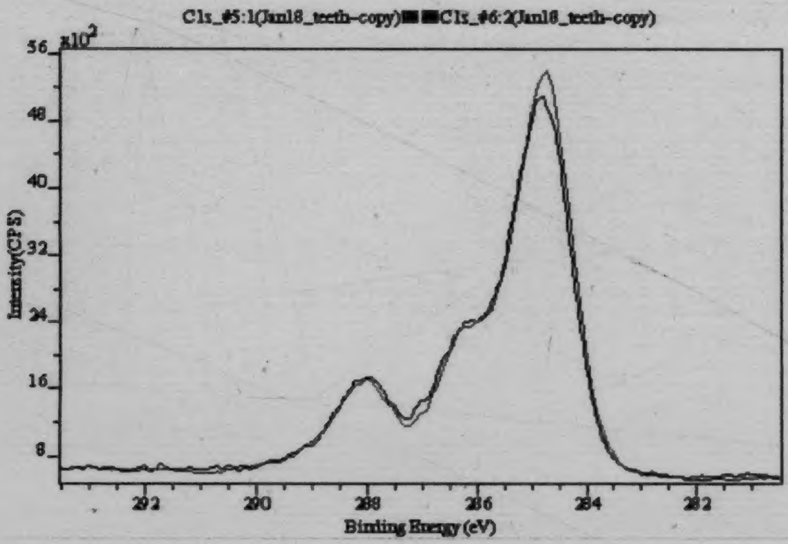


Figure 2.4a-j Scatter plots of the main elements: Ca, P, O, C and N

Figure 2.4a Scatter plot of Ca to P

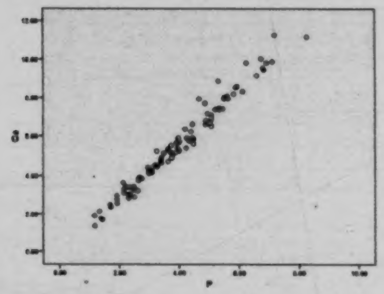


Figure 2.4b Scatter plot of Ca to O

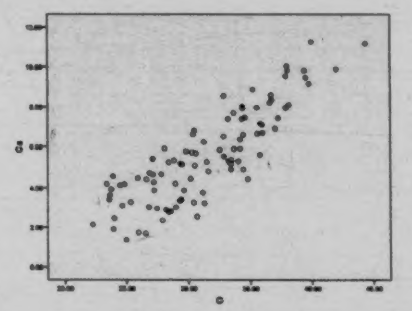


Figure 2.4c Scatter plot of P to O

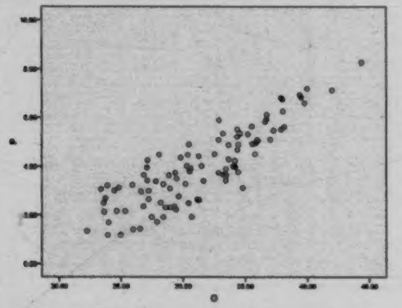


Figure 2.4d Scatter plot of C to N

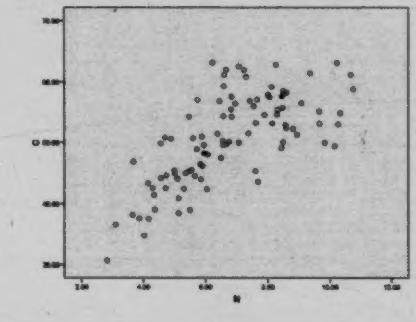


Figure 2.4e Scatter plot of Ca to C

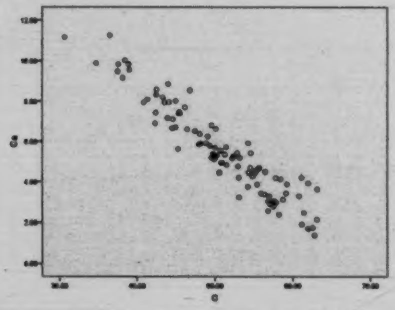


Figure 2.4f Scatter plot of Ca to N

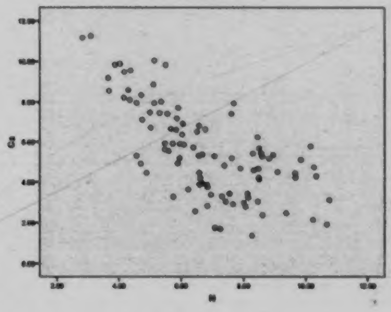


Figure 2.4g Scatter plot of P to C

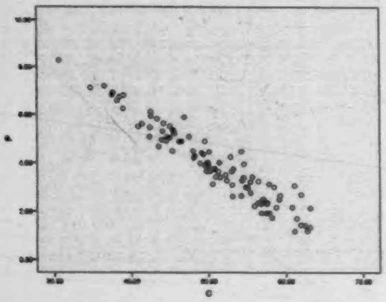


Figure 2.4h Scatter plot of P to N

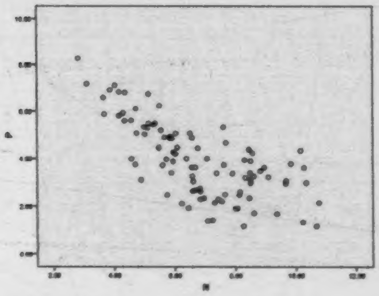


Figure 2.4i Scatter plot of O to C

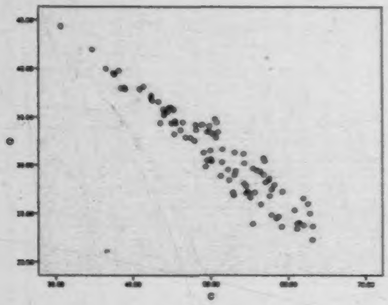
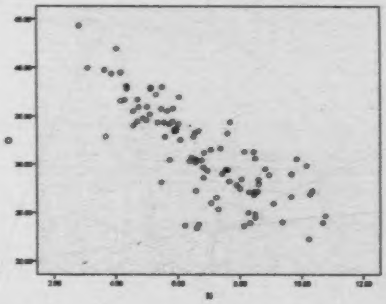


Figure 2.4j Scatter plot of O to N



Scatter plots of the main elements showed that as carbon increased so did nitrogen which indicated a positive relationship between carbon and nitrogen. Calcium, phosphorus and oxygen also showed a positive relationship to each other. However, as carbon and nitrogen concentrations increased, calcium, phosphorus and oxygen concentrations decreased indicating a negative relationship between these elements (Figure 2.4a-j). To find out if there is a difference in the chemical composition between the buccal surfaces of the maxillary right and left first premolar (Appendix F – raw data) paired t-test was conducted which found no statistical significant difference in any of the chemical composition between the right and left first premolars (p>0.05). All statistics analyses were performed using SPSS software (SPSS 15.0, Chicago, IL) and can be found in Appendix G.

2.4 DISCUSSION

A typical survey spectrum of the right and left maxillary first premolar from the same subject is shown in Figure 2.2. The peaks were labeled with the abbreviated name, followed by the quantum number of the ejected photoelectron. The area under the peaks facilitated the quantification of the relative amounts of each element present, while the shape and position of the peaks represented the chemical state of each element. The main peaks detected were Ca 2s, 2p, P 2s, O 1s, C 1s and N 1s, while other smaller peaks represented elements in minor concentrations.

The result of this study agreed with other studies in that Ca, P, O, C and N were the main elements found in enamel.[14, 15] It is however difficult to make direct comparison of the current study to these studies as the chemical compositions were reported in percentage and the total number of elements examined were different between the studies. Therefore, unless the studies examined the same number of elements in the studies, the proportionality of each element will be affected. Further, Weatherell *et al.* [16] has shown that the surface of the human enamel is very heterogeneous with wide and rather irregular variations from location to location and from tooth to tooth. Unless the studies examined enamel from the same location and from the same type of tooth, comparisons of the chemical compositions may be invalid.

When visual comparisons were made of all the survey and high resolution scans between the right and left maxillary first premolar enamel, very little difference in appearance of the scans was noted. This was supported by the statistical analysis which showed no significant difference ($p = 0.457$) in any of the chemical composition between the maxillary right and left first premolar. This finding was expected as the eruption of the same type of corresponding tooth is within the time frame of about six months.[17] During a particular period of time of tooth eruption, the teeth are exposed to the same chemical environment of the oral cavity which should result in similar ions and chemicals being incorporated into enamel.

For the major elements detected on the enamel surface, Ca, P and O were found to have a positive relationship to each other, that is, as one element increased, so did the other two elements (Figure 2.4). This was expected as Ca, P and O are the constituents of hydroxapatite crystals, which has a formula of $Ca_{10}(PO4)_6(OH)_2$. Carbon and nitrogen were also shown to have a positive relationship to each other. This was also expected as carbon and nitrogen are the main components of amino acids which make up the proteins found in the enamel. Since enamel is made from 96 wt % inorganic mineral, 3 wt % water and about 1 wt % organic materials of proteins and lipids, as the inorganic minerals, namely Ca, P and N increase, the organic components, namely N and C will be displaced and decreased. This was seen in Figure 2.4 as the negative linear relationship between Ca, P, O which made up the hydroxyapatite and C and N which constituted the protein. [18, 19]

Table 2.2 Calcium to phosphorus ratio of buccal section of maxillary first premolar

Buccal enamel	Sample Size	Minimum	Maximum	Mean	SD
Ca to P ratio	98	1.16	1.71	1.4101	0.10448

Based on the pure hydroxyapatite crystal formula of $Ca_{10}(PO4)_6(OH)_2$, the stoichiometric ratio of Ca to P is approximately 1.67. Due to impurities present in enamel, the Ca to P ratio has historically been in the range of 1.48 ± 0.09.[20] The ratio obtained in the current study was about 1.41 and was consistent in this range (Table 2.2). Since the Ca to P ratio of biological hydroxyapatite has always been observed to be lower than its pure form, the term "calcium-deficient" apatites has sometimes been used to describe enamel apatites. Chusuei *et al.*[21] explained this apparent decrease in the ratio as due to the instability of hydroxyapatite when it was exposed to the X-ray source leading to selective ejection of calcium ions by XPS. Most other researchers[20, 22]explained this decrease in ratio as due to adsorption of excess phosphates on the crystal surfaces, substitution of calcium by sodium and magnesium, or the incorporation of impurities or trace elements. Regardless of the possible explanations for the decrease in Ca to P ratio, biological apatites are generally considered as non-stoichiometric.[22]

For the element with the highest atomic concentration, carbon was further analyzed to obtain its components. Carbonate was of particular interest as high carbonate content has been shown to increase enamel to caries susceptibility.[23, 24] The mean carbonate content obtained in the current study was 3.01% (Table 2.1) which agreed with the finding of Sydney *et al.*.[25] Further, no statistically significant difference ($p = 0.288$) was found between the carbonate content of right and left maxillary first premolar. The carbonate to phosphorus ratio was also calculated (Table 2.1) as it is believed that a ratio is associated with an increase in the susceptibility of enamel to acid attack.[13]

The mechanism of the effect of carbonate on the chemical instability of the mineralized enamel apatite has been postulated.[26, 27] It is believed that the effect of carbonate on the structural properties of the apatites is through a reduction in apatite crystal size, an increase in apatite crystal strain, and a substitution of a weaker Ca-CO_3 bond for a stronger Ca-PO_4 bond. Thus, carbonate has a profound effect on the chemical and physical properties of enamel. Through the disruption of the crystal lattice structure by carbonate, a carbonated-apatite becomes more susceptible to acid attack than pure hydroxyapatite.[28]

If the high carbonate content or high carbonate to phosphorus ratio in the enamel predisposed it to carious attack, it is not clear if they would also predispose the enamel to yield a potentially better etching result when an acid etchant is applied to the enamel surface prior to orthodontic bonding of brackets to enamel surface. With a potentially better etch quality and etch pattern there may be an improvement in the bond strength. On the contrary, fluoride incorporated into the hydroxyapatite has been shown to impart higher level of resistance to acid attack.[29, 30, 31, 32] It is not clear if this higher level of resistance to acid attack would result in a less ideal etching and a possible decrease in bond strength.

To date, there has been no published report to support the above statements, but only anecdotal reports from clinicians. Since the current study found no statistically significant difference between any of the chemical elements of the right and left maxillary first premolars, future research based on the current data will involve randomly dividing the enamel samples into an *in vitro* bonding study and a qualitative etching pattern study. The results of the bonding and qualitative etching studies can assist us in the understanding of the inter-relationship between enamel chemical composition, etching pattern and bond strength.

2.5 CONCLUSIONS

1. There was no statistically significant difference between the chemical composition of the maxillary right and left first premolar ($p > 0.05$). All of the 14 p-values based on paired t-tests were > 0.05.
2. The major elements detected in the enamel samples were calcium, phosphorus, oxygen, nitrogen and carbon.

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Chapter 3

Chemical Composition of Enamel Surface as a Predictor of *in vitro* Shear Bond Strength

3.1 INTRODUCTION

Bonding of orthodontic attachments to enamel surface is now a widely accepted technique due to the ease of bonding and a reasonable success rate. Bond failure however, does occur and varies according to different clinicians.[1,2] Many theories have been proposed and studied, including surface topography of enamel[3], operator technique[4], masticatory forces in various regions of the oral cavity[5], and the different adhesive and bracket systems used in bonding.[6,7] One area that has received minimal attention is the possible variation in the chemical composition of the enamel surface as a contributor to the large variation of bond strength and failure frequently seen in the clinical and laboratory settings.

In the in vitro bonding literature, it is well known that bonding studies are notorious for producing results with large variances.[8,9,10,11] This large variability in the bonding results has led researchers to believe that bond strength depends not only on the intrinsic performance of the adhesive-composite system but also on the chemical profile of the enamel surface.[12]

With advances in technology, the chemical composition of the enamel surface can now be studied using x-ray photoelectron spectroscopy (XPS) without altering or destroying the enamel samples. The non-destructive nature of the technique permits further studies such as *in vitro* bonding or etching pattern analyses and the determination of the inter-relationship between the collected data.

The chemical composition of the buccal enamel surface of 98 maxillary right and left first premolars was determined using XPS in a previous study (Chapter 2). Since no statistically significant difference (p>0.05) was found between the chemical composition in the right and left maxillary first premolars, half of the enamel samples was allocated to an *in vitro* bonding study and the remaining to a qualitative etching pattern study. The focus of this study was to relate the chemical composition to the *in vitro* bond strength.

3.2 MATERIALS AND METHODS

After ethics approval from the Heath Research Ethics Board of the University of Alberta (Appendix A), the recruitment of patients from the Orthodontic Graduate Clinic at the University of Alberta was initiated (Appendices B and C). Extracted human maxillary first premolar tooth was selected for use in the study because these teeth are the most common teeth to be extracted during orthodontic treatment. Teeth with craze lines, cracks, demineralization and previously restored buccal surfaces were excluded from the study. A total of 51 patients were recruited for the study, but 2 patients were excluded as their enamel samples were fractured during the transport process to the engineering building for XPS analysis. In the end, a total of forty-nine patients requiring the extraction of maxillary right and left first premolars as part of the comprehensive orthodontic treatment met the study inclusion criteria which resulted in a total of 98 enamel samples.

After extraction, the teeth were wiped clean with gauze to remove tissue debris and each stored in a container with 0.1% thymol solution to prevent bacterial and fungal growth. The containers were labeled with a code so the investigators were blinded with regards to the identity of the teeth. At day 14, the teeth were rinsed with distilled water prior to sectioning the buccal portion of the enamel longitudinally in a mesio-distal direction from the lines angles with a diamond disc (Brasseler Dental Instrumentation, Savannah, Georgia, USA). The sectioned enamel samples were rinsed with copious distilled water and stored separately in a pill organizer. When 8 enamel samples have been obtained the chemical composition of the samples was analyzed using XPS through the Alberta Center for Surface Engineering and Science (ACSES) at the University of Alberta. The operational details of the XPS were described in Chapter 2.

After the chemical composition of all the 98 enamel samples have been analyzed, a preliminary statistical analysis was performed which indicated no significant difference in the chemical composition ($p>0.05$) between the right and left maxillary first premolars. Since there was no statistical significant difference in the chemical composition, the enamel samples were randomly divided equally into an *in vitro* bonding study group and a qualitative etching pattern study group using a randomization table generated by a statistician (Appendix H).

For the *in vitro* bonding study, the buccal surface of the enamel surface was etched with 40% ortho-phosphoric acid gel (Patterson Brand, St. Paul, MN, USA) for 30 seconds. The etched surface was then rinsed with a combination of air and water spray and dried with an oil-free air source for 5 seconds until the buccal surfaces of the etched teeth appeared to be chalky white in color. The surface was coated with a Transbond XT Light Cure Adhesive Primer (3M Unitek, Monrovia, CA. USA) and a universal premolar type adhesive pre-coated Victory Series metal bracket (3M Unitek, Monrovia, CA, USA) with a calculated surface area of 12.18mm^2 bonded onto the center of each enamel surface. Excess adhesive was removed and the bracket light cured with Ortholux LED curing light (3M Unitek, Monrovia, CA, USA) for 20 seconds from the mesial and 20 seconds from the distal, for a total of 40 seconds. After all the enamel samples were bonded with a bracket, each of the enamel samples was embedded with Duralay (Reliance, Dental Mfg. Co.,Worth, IL, USA) in a square aluminum block. A mounting jig (Figure 3.1) and a standardized wire of 0.0215" x 0.028" was utilized to align the buccal surfaces of the teeth perpendicular with the bottom of the aluminum block (Figures 3.2 and 3.3) so that the labial surfaces would be parallel to the applied force during the shear test.

Figure 3.1 Customized mounting jig for embedding the enamel-bracket sample

Figure 3.2 Addition of Duraly to the enamel-bracket sample

Figure 3.3 Enamel-bracket sample embedded in Duralay ready for shearing test

After each enamel sample was embedded in Duralay, the enamel sample identification number was transferred to the aluminum square to ensure future identification in the debonding study. When all the samples have been embedded in Duralay, thermocycling was carried out using the technique described by Lee-Knight *et al.*[13] between 2 water baths containing distilled water at 55° Celsius and 5° Celsius. Seven hundred and fifty cycles were performed between these two temperatures with a dwell time of 30 seconds.

After all the samples have been thermocycled, the *in vitro* bonding study was carried out to determine the shearing load required to cause bracket removal using a MTS, Synergie 400 (MTS Systems Corporation, Eden Prairie, USA) machine with a load cell of 500N and a measurement error of 0.003% of the full scale (1.5N). A blunted stainless steel rod was fixed in the upper grip face, and a mounting jig in the lower grip face, into which the aluminum square block was placed. The cross-head speed was set at 1 mm/minute, with the direction of force application parallel to the bracket base in an occluso-gingival direction. The rod end was placed between the bracket tie-wings and the bracket pad, as close to the tooth as possible (Figures 3.4 and 3.5).

Figure 3.4 MTS Machine	Figure 3.5 Closer view of MTS machine

Each enamel sample in the aluminum square block was placed into the mounting jig apparatus of the MTS machine, and the stainless steel cross-head rod was lowered into position between the bracket base and tie-wings to be just slightly out of contact with the bracket prior to testing. A computer connected to the MTS machine, using the Testworks™ program (MTS Corporation, Eden Prairie, USA) controlled the crosshead speed, recorded the peak load in Newtons and peak stress in MPa at bracket failure.

All the bonding procedures, thermocycling and shearing test were performed by a single operator in order to eliminate inter-operator variability.

3.3. RESULTS

Twelve elements were detected in the buccal surface of enamel surface (see Chapter 2) but there was no significant difference in the chemical composition between the right and left maxillary first premolars ($p>0.05$). The *in vitro* shear bond strength had a mean of 6.9347 MPa ± 2.71436 MPa (Table 3.1). Based on adhesive remnant index score, there were no samples that had the entire adhesive left either on the enamel surface or the bracket base (Table 3.2). The raw data for the bonding study can be found in Appendix I.

Table 3.1 Descriptive statistics of mean shear bond strength measured in megapascal (MPa)

	Sample size	Minimum	Maximum	Mean	SD
Bond strength	49	2.90	12.30	6.9347	2.71436

Table 3.2 Adhesive Remnant Index (ARI) showing the site of bond failure

Adhesive Remnant Index Score				
	0	1	2	3
Counts	0	26	23	0
Percentage	0	53.06	46.94	0

0 = no adhesive on tooth
1 = less than 50% adhesive on tooth
2= more than 50% adhesive on tooth
3 = all adhesive remain on tooth

Since magnesium and chlorine were found in only a limited number of the enamel samples, they were not included in the multiple linear regression analysis. In addition to the 10 elements, carbonate, and carbonate / phosphate ratio were used as predictors in the backward multiple linear regression models to predict the *in vitro* bond strength. Eleven multiple linear regression models were generated (Appendix J). When all 12 predictors were included, R^2 of 33.3% was obtained but none of the 12 predictor variables were found to be significant ($p=0.170$). Backward selection removed the insignificant variables which resulted in only calcium and phosphorus as significant predictors ($p=0.008$) of *in vitro* bond strength with R^2 of 18.8%. However, bivariate scatter plot of calcium and phosphorus indicated a significant co-linearity (0.982 at a p value of 0.0001) as measured by tolerance and variance inflation factor (VIF) which is a violation of multiple linear regression as it will cause instability of the regression coefficients resulting in a lack of precision in estimating the regression coefficient. Therefore, calcium and phosphorus were separated into two models and each run independently of the other element in simple linear regression. Calcium was able to explain the variation in the *in vitro* mean bond strength with R^2 of 0.9% and the contribution was not significant ($p=0.526$); phosphorus was able to explain the variation in the *in vitro* mean bond strength with R^2 of 3% and the contribution was not significant ($p=0.237$). Carbonate as the sole predictor of mean bond strength explained 2.4% of the variations observed in the mean shear bond strength and the contribution was not significant ($p=0.288$). None of the 12 variables was able to significantly explain the *in vitro* mean bond strength. Post-hoc power analysis with R^2 of 33.3% showed 80% power for the current study (Appendix K). All statistical analyses were performed using SPSS software (SPSS 15.0, Chicago, IL) and can be found in Appendix J.

3.4 DISCUSSION

The *in vitro* shear bond strength of the current study has a mean of 6.93 ± 2.71 MPa. The result agreed with the studies by Bishara *et al.*[7, 14] who conducted *in vitro* shear bond strength using the same type of adhesive precoated brackets and adhesive. There was a wide range of bond strength noted in this study ranging from 2.90 to 12.30 MPa. This was also in agreement with many of the published studies on *in vitro* bonding which found [7, 8, 9, 10, 11] a wide variation in the magnitude of the bond strength.

From a clinical perspective, the wide range of bond strength values is concerning. Various studies have suggested bond strengths ranging from 2.8MPa to 10 MPa as being adequate for clinical situations[15, 16] but a review of these studies did not reveal how these values were determined except the authors acknowledged that it is difficult to determine the mean bond strength required to withstand the occlusal force. Since there is no scientifically determined ideal bond strength range, there must be a value below which brackets will fail with normal masticatory forces. Rather than reporting and using mean bond strength, perhaps a more meaningful measure of an adhesive's value is the proportion of bonds that are above the threshold value. If a large proportion of values are at the low end of the distribution, it can be inferred that a large number of bonds will likely fail in the clinical setting. Future research in determining the clinically acceptable minimum bond strength value would greatly improve the validity of conclusions drawn from bonding studies.

Regardless of the bonding material used, a range in bond strengths will result. Many theories have been proposed to explain this phenomenon and one of them was the role of chemical composition on bond strength.[12, 17, 18] Statistical analysis using regression analysis, however, did not support the role of chemical composition as a significant factor in the *in vitro* mean shear bond strength. None of the detected elements were significant predictors of mean bond strength.

In the literature, carbonate has been coined as the Achilles' heel of dental enamel as enamel rich in carbonate has been found to be particularly susceptible to acid attack[19] and a high carbonate-to-phosphate ratio has been associated with teeth particularly susceptible to acid attack.[20] It is hypothesized that the variations in the carbonate content on enamel surface could affect enamel's susceptibility to intentional acid etching of the premolars prior to orthodontic bonding. This variation may affect the amount of micro-porosity created which is necessary for a superior mechanical bonding.

The relationship between the chemical composition of human enamel and the quantitative surface area of the porosity created by the acid etching was examined by Orellana *et al.* using the technique of argon gas adsorption[19] The study utilized argon gas in a pressurized environment to coat the surface of the etched enamel in order to quantify the surface area of the micro-porosity created by the acid etching process. The results of the argon gas adsorption indicated that carbonate on the enamel surface was not a significant predictor of the etched surface area which may explain the finding of the current study where carbonate when used as a sole predictor of mean bond strength explained only 2.4% of the variation in mean bond strength and the contribution was not significant ($p = 0.288$). Factors other than chemical composition therefore, must be responsible for the variation seen in the *in vitro* mean shear bond strength.

A search of the literature database that studied the relationship between chemical composition of enamel and *in vitro* mean bond strength yielded only a single study. This study[12] found a positive correlation between enamel hardness and *in vitro* bond strength and a positive correlation between hardness and calcium concentration. The authors therefore concluded that calcium was a significant determinant of *in vitro* mean bond strength. The validity of the study by Panighi *et al.*[12]however, is questionable. In the study by Panighi *et al.*[12] non-specified human molars were used in conducting the two independent experiments and only the content of two elements, calcium and phosphorus were analyzed. There was no statistical or regression analysis of the data to quantify the significance of the study. Panighi *et al.*[12] related calcium as responsible for the enamel hardness and through enamel hardness indirectly related calcium concentration as a determinant of bond strength. However, enamel hardness is a composite of several parameters, with several factors contributing to it.[18] Baud and Lobjoie[21] suggested that the arrangement of the surface crystallites was responsible for the hardness of enamel, while the water content of enamel has been suggested by Brudevold *et al.*[22] and Carlstrom *et al.*[23] as determinant of enamel hardness. In addition, Weatherell *et al.*[24] was not able to find a high degree of correlation between enamel hardness and the percentage of calcium which contradicted with the results of Panighi *et al.*[12] The use of the buccal surface of the molar for the *in vitro* bonding study by Panighi *et al.*[12] also raised question as the buccal surface of molars usually results in poor adaptation of orthodontic bracket to the surface leading to higher polymerization shrinkage of adhesive and variable bond strength.[25]

In the current study, the percentages of calcium and phosphorus were determined and their relations to *in vitro* mean shear bond strength were quantified with regression analysis. Calcium explained only 0.9% of the variations observed in the *in vitro* mean shear bond strength and the contribution was not significant (p=0.526); phosphorus explained only 3% of the variations noted in the current study and the contribution was not significant (p=0.237). Post-hoc power analysis was performed using R^2 of 33.3 % and indicated power of 80%. With a power of 80% and the current study carried out using a large sample size of 49 enamel samples, there was no evidence to indicate that the chemical composition was a significant predictor of *in vitro* shear bond strength. Other factors, therefore, must play an important role in variations of the bond strength.

An interesting observation was noted while preparing the enamel samples for the bonding study which may contribute to the large variances common to many *in vitro* bonding studies. After the buccal surfaces of all the enamel samples were sectioned from the mesio-buccal to the disto-buccal line angles with a diamond disc, the variation in the convexity of the buccal enamel surface was visually more apparent than before the surfaces were sectioned from the remaining tooth. It is possible that when looking at an entire tooth, there were too many distracters which may mask the variation in the shape of the buccal enamel surface. However, when the buccal surface was sectioned and viewed visually in isolation, the variation was easier to identify.

While it is recognized that variation in the convexity of enamel surface may result in a poor adaptation to the base of the brackets[25] which currently are all manufactured from the same mold for a given tooth type, it is not clear how much of the variation is needed to have an effect on the bond strength. With the technology available, it may be possible to use laser to scan the buccal surface of the enamel surface and bracket base and determine the closeness of the fit using custom-made computer software. This will allow quantification of the differences which may play an important role in the large variances frequently noted in laboratory bonding studies.

The results of the current study indicated that the chemical composition was not a significant predictor of *in vitro* mean bond strength. It is recommended that future research examine the shape of the enamel bonding surface as a potential source contributing to the large variances in laboratory bonding study. The manufacturers of orthodontic brackets are encouraged to custom made the bracket base to the individual contour of the bonding surface to gain a competitive edge if it is determined that the contour of the bonding surface is critical for a superior bond.

3.5 CONCLUSIONS

1. The chemical composition of the buccal surface of maxillary first premolar was not significant in predicting the *in vitro* mean shear bond strength. When the 10 detected elements and CO_3^- and CO_3^- / P ratio were included as predictors, R^2 of 33.3% was obtained. However, none of the chemical composition variables was significant ($p=0.170$).
2. Carbonate explained only 2.4% of the variations observed in the *in vitro* mean shear bond strength and the contribution was not significant ($p=0.288$).

3. Calcium explained only 0.9% of the variations observed in the *in vitro* mean shear bond strength and the contribution was not significant (p=0.526).
4. Phosphorus explained only 3% of the variations in the *in vitro* mean shear bond strength and the contribution was not significant (p=0.237).

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CHAPTER 4

THE EFFCT OF ENAMEL CHEMICAL COMPOSITION ON THE QUALITATIVE ETCHING PATTERN AND THE INTER-RELATIONSHIP BETWEEN ETCHING PATTERN AND *IN VITRO* SHEAR BOND STRENGTH

4.1 INTRODUCTION

Bonding of orthodontic attachments to enamel surface requires the preparation of enamel surface through acid etching prior to adhesive placement. The etching process exposes the enamel prisms and creates the micro-porosity necessary for the creation of a quality mechanical adhesion.[12]. The resulting qualitative etching patterns have been studied and categorized by many researchers[2, 3, 4, 5, 6, 7]. The differences in etching patterns have been explained by some researchers as a result of the various apatite crystal orientation on the enamel surface and the difference in chemical profile of the enamel surface[8, 9]. The classification of the enamel etching pattern has important clinical implication as it has been anecdotally reported by McLaughlin[10] that a good quality etch was a primary determinant in the production of a quality mechanical bond between orthodontic attachment and enamel surface. To date, there has been no published study that simultaneously examined the inter-relationship of enamel chemical composition, qualitative etching pattern and the resulting shearing bond strength.

The current study was undertaken to determine the qualitative enamel etching pattern of maxillary first premolars using scanning electron microscope (SEM). This information was then related to the enamel chemical composition as determined using X-ray photoelectron spectroscopy (XPS) in a previous study in chapter 2. Since the etching pattern has been anecdotally reported to affect orthodontic bond strength[10], the potential relationship between the qualitative etching pattern and orthodontic shear bond strength established in a previous study in chapter 3 was also investigated.

4.2 MATERIALS AND METHODS

After ethics approval from the Heath Research Ethics Board of the University of Alberta (Appendix A), the recruitment of patients from the Orthodontic Graduate Clinic at the University of Alberta was initiated (Appendices B and C). A total of forty-nine patients requiring the extraction of maxillary right and left first premolars as part of the comprehensive orthodontic treatment met the study inclusion criteria which resulted in a total of 98 enamel samples.

The chemical composition of all the 98 enamel samples was analyzed using x-ray photoelectron spectroscope (XPS). The operational details of XPS and the results of the study were covered in chapter 1. Since it was determined in chapter 2 that there was no significant difference in the chemical composition ($p > 0.05$) between the right and left maxillary first premolars, the enamel samples were randomly divided equally into an *in vitro* shear bonding study group and a qualitative SEM etching pattern study group using a randomization table generated by a statistician (Appendix H). The operational details and the results of the *in vitro* shear bonding study can be found in chapter 3.

For the qualitative SEM etching pattern study of the enamel, the buccal surface of 49 enamel samples was etched with 40% ortho-phosphoric acid gel (Patterson Brand, St. Paul, MN, USA) for 30 seconds. The etched surface was then rinsed with a combination of air and water spray and dried with an oil-free air source for 5 seconds until the buccal surfaces of the etched teeth appeared to be chalky white in color. The etched enamel samples were then baked dry in an oven at 50°C for 2-3hr and mounted in aluminum stub before being coated with a 30Å layer of gold in vacuum using Edwards High Vacuum Sputter Coater S105B (Manor Royal, Crawley, West Sussex, RH 102LW, England) to prevent charge build-up on the specimen during electron bombardment. After sample preparations, the specimens were examined using a Hitachi S-2500 scanning electron microscope (Hitachi, Tokyo, Japan) operating at an accelerating potential of 4-8 kV with a 0° specimen tilt angle and an operating pressure of 10^{-4} mmHg.

The enamel samples were first viewed under 50X magnification to locate the mid-buccal portion of the sample which represented the orthodontic bonding area. The samples were then imaged under 500X, 1500X and 3000X magnifications. The determination of the qualitative etching pattern was based on the pattern that occupied the majority of the bonding surface as viewed under 1500X magnification SEM image using a modification of the Galil's etching classification as proposed by Hobson *et al.*[7]:

Type 1 – well-developed conventional etch pattern with well-defined prisms
Type 2 - discernible prisms apparent but poorly defined
Type 3 – no prism definition but surface roughening has occurred
Type 4 – flat smooth surface

After 3 months had elapsed, a second attempt at the determination of the qualitative etching pattern was performed based on the 1500X SEM image by the same operator to establish the intra-rater reliability. All the etching procedures and qualitative etching patterns were performed by the same operator in order to eliminate inter-operator variability.

4.3 RESULTS

The raw data of the inter-relationship between the qualitative acid etching pattern and the enamel chemical composition of the labial surface of maxillary first premolar is shown in Appendix L. The data for the relationship between qualitative acid etching pattern and *in vitro* shear bond strength can be located in Appendix M. All statistics accompanying this chapter can be found in Appendix N.

Table 4.1 Frequency distribution of qualitative enamel acid etching pattern

Etching pattern	Frequency	Percent	Mean bond strength (MPa)
Type 1	10	20.4	7.07
Type 2	9	18.4	6.96
Type 3	19	38.8	6.93
Type 4	11	22.4	6.81

Figure 4.1 Frequency distribution of enamel acid etching pattern

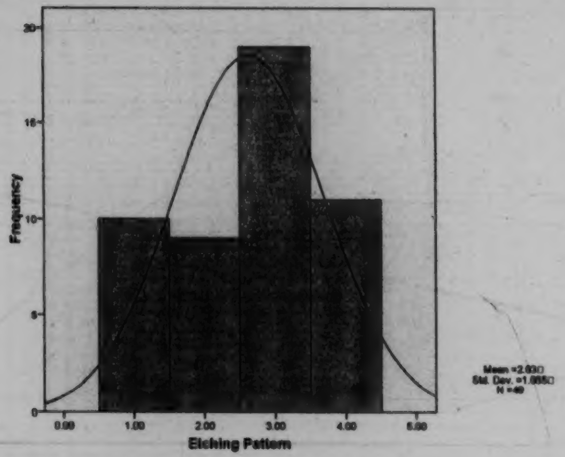


Table 4.1 and Figure 4.1 revealed Type 3 etching pattern was the most frequently observed etch pattern. The two attempts on etching pattern identification at an interval of 3 months by the same operator showed an intraclass correlation coefficient (ICC) of 0.946. The illustrative examples of Type1, 2, 3, and 4 etching patterns can be found in Figures 4.2 – 4.5 (the comprehensive SEM images of all the samples are included in a CD). Using regression analysis, the chemical composition of the enamel surface was able to explain 20.8% of the variation observed in qualitative etching pattern but none of the chemical composition variables were significant (p= 0.40). The qualitative etching pattern explained 0.1% of the variation in shear bond strength but the contribution was not significant (p= 0.832).

Figures 4.2-4.5: Illustrative examples of Type 1, 2, 3, and 4 etching patterns

Type 1 Etching Pattern

Sample AH8-24B (X3000)

Sample AH8-24B (X1500)

Sample AH8-24B (X500)

Type 2 Etching Pattern

Sample KH15-14B (X3000)

Sample KH15-14B (X1500)

Sample KH15-14B (X500)

Type 3 Etching Pattern

Sample BE63-14B (X3000)

Sample BE63-14B (X1500)

Sample BE63-14B (X500)

Type 4 Etching Pattern		
 Sample RE50-24B (X3000)	 Sample RE50-24B (X1500)	 Sample RE50-24B (X500)

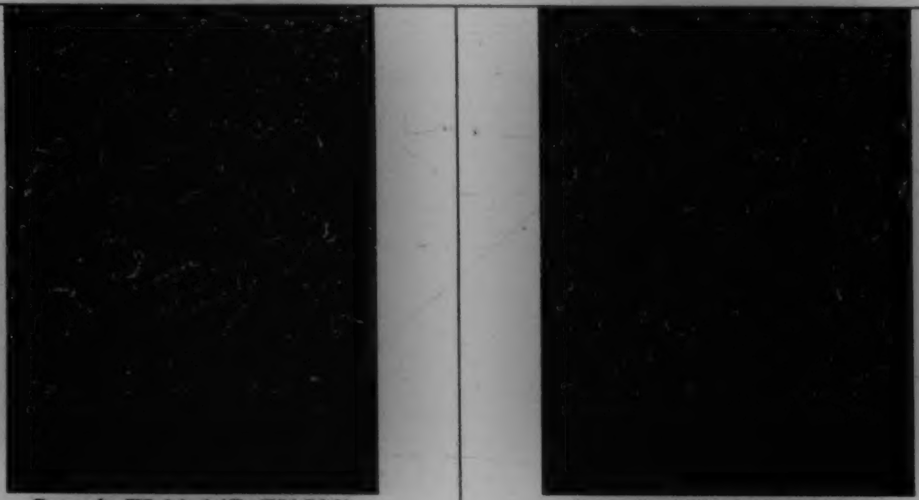
4.4 DISCUSSION

The most frequently observed qualitative etching pattern in this study was Type 3, denoting surface roughening without prism formation. This finding did not agree with the results of previous published studies[6, 11, 12, 13, 14, 15] on SEM etching patterns from the 1970 and 1980's which reported mainly ideal Type 1 etching patterns. A careful review of these previous studies revealed that the researchers have ground the enamel surface prior to acid etching which removed the more acid-resistant prismless enamel and exposed the more acid-susceptible underlying surface for etching[6, 11, 12, 13, 14, 15]. Grinding of enamel surface before orthodontic bonding is not a commonly done procedure therefore the resulting etching pattern is not a true reflection of what takes place in a clinical setting. The result of the current etching pattern study however, agreed with the findings of more recent SEM etching studies which utilized large enamel samples without surface grinding and a systematic method of evaluating the acid etch pattern occupied in the orthodontic bonding area[7, 16, 17].

The current study also found that the chemical composition of the buccal enamel surface was able to explain 20.8% of the variation observed in qualitative etching pattern but the contribution was not significant (p= 0.40), suggesting that other factors may be responsible. Johnson *et al.*[8] postulated the role of enamel crystal apatite orientation as a determining factor in the differential etching patterns. It was suggested that different faces of individual enamel crystals exhibited differences in the reactivity to acid etchant. Studies by Johnson and Sharpe[18, 19] indicated that enamel crystals dissolved in acid more quickly along the c-axes than perpendicular to this axis. Another possible explanation for the non-significance of the relationship between chemical composition and etching pattern in the current study may be the inadequacy of the existing method used in classification of the qualitative etching pattern.

The existing method of enamel acid etching pattern classification is based on a rather subjective qualitative method. The etch pattern that predominated in the etched area when viewed under SEM constituted the etch pattern. However the etching pattern in enamel has been shown to be very heterogeneous both in this study and many published reports[5, 7, 16, 20, 21, 22]. Frequently, areas with pronounced, well defined etch alternated with poorly defined areas within the same tooth surface on the orthodontic bonding area (Figure 4.6). Instead of a 100% Type 1 etched pattern, it was common to find that different etch patterns were mixed in a given area. This is to say that a Type 1 etching pattern is not truly 100% Type 1 and a Type 2 etching pattern is not truly 100% Type 2 etching pattern and so forth. This inadequacy in the etching classification has certain clinical implication.

Figure 4.6 Both samples were classified as exhibiting Type 1 etching patterns as ideal etching patterns occupied the majority of the etched surface.~~—But~~ sample on the right exhibited more extensive Type 1 pattern



Sample EL11-14B (X1500)

Sample AH8-14B (X1500)

Under the current qualitative etching pattern classification, the etch pattern that occupied the majority of the etched surface constituted the etch pattern. That is, an enamel with a 1% poor etch and a 99% ideal etch would be classified as Type 1 pattern. However, an etched enamel surface with a 25% poor etch and a 75% ideal etch would also be identified as Type 1 pattern. These two Type 1 etched enamel samples clearly have different etch characteristics, however under the current classification system, they are both classified as having the same etch pattern. In essence, the current enamel etching pattern classification is overly simplistic.

If the assumption by McLaughlin[10] that an ideal etch pattern is necessary for a good mechanical bond strength between an orthodontic attachment and the etched enamel surface, then the current over-simplistic qualitative etching pattern may also explain the lack of significant regression (p= 0.832) relationship found in this study between qualitative etching pattern and *in vitro* shear bond strength as indicated by a R^2 of 0.1%. That is, the quality of the retention cannot be determined by evaluating the structure of the etched enamel surface under the existing method.

Despite the inadequacy of the existing qualitative enamel acid etchant classification, the intra-rater reliability of two repeated attempts in etching pattern identification based on the existing 1500X SEM images with an interval of 3 months apart showed a high intra-class correlation of 0.946. The high intra-rater reliability merely indicated a high degree of consistency in identification, but it is not an endorsement of its suitability or appropriateness of the method in predicting *in vitro* bond strength. An alternative or an improvement in the current qualitative enamel etching classification is needed to characterize the etched enamel surface.

In an effort to improve the existing qualitative etching pattern classification, Hobson[7] utilized a semi-quantitative etching classification using SEM. With this method, a grid with 30 intersection points was superimposed on the displayed SEM image and moved horizontally and vertically into 64 different regions of the bonding area providing a total of 1920 intersections for systematic evaluation. The number of each etching types was recorded and expressed as a percentage. An etched enamel surface would therefore be described as composed of a percentage of Type 1, 2, 3 and 4 patterns. Hobson postulated that the percentage of each etching types that occupied the bonding area was an important determinant of the bond strength. However, in a subsequent study, Hobson and

McCabe[23] was unable to find a significant relationship ($p = 0.504$) between the proportion of ideal etch and bond strength.

Brannstrom *et al.*[4] suggested the lack of ability of enamel etching pattern as observed under SEM to predict the *in vitro* bond strength was due to the shortcoming of the SEM itself. The operation of the SEM is based on the ability of electron beam to scan the acid etched surface. However, electron beam cannot reach deep into narrow depressions and channels in the etched enamel. The qualitative etching pattern as seen under SEM is merely a visual appearance of the effect of acid etchant and is a poor indicator of the volume of surface irregularities on the enamel which is a better predictor of retention. Gunadi and Nakabayashi[24] agreed with Brannsstrom that the resin-enamel bond strength is the result of the cumulative cross-sectional area of etched enamel porosity and not the formation of well-defined etched patterns.

With the limitation of the existing qualitative enamel etching pattern classification and the questionable validity of SEM as an intermediate tool in determining bond strength, the development of a quantitative technique in studying the etched enamel surface is warranted. A quantitative method of analyzing the etched enamel surface has recently been proposed and subsequently developed by Orellana *et al.*[25]. This technique utilized argon gas in a pressurized environment to coat the surface of the etched enamel in order to quantify the surface area of the micro-porosity created by the acid etching process. It will be beneficial in the future to determine the minimal surface area created by the etching process required for adequate clinical bond strength so the irreversible loss of enamel through etching process can be minimized. It is likely that the development and future refinement of this quantification method of evaluating the etched enamel surface will enhance our understanding of the relationship between acid etching and bond strength.

4.5 CONCLUSIONS

1. The most common enamel etching type was Type 3, denoting a surface where roughening has occurred but no clear prism definition was evident
2. The chemical composition of the enamel surface was able to explain 20.8% of the variation observed in qualitative etching pattern but the contribution was not significant (p=0.40).
3. The qualitative etching pattern explained 0.1% of the variation in shear-peel bond strength but the contribution was not significant (p= 0.832).

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CHAPTER 5 DISCUSSION

5.1 FOCUS OF PROJECT

This research was carried out using 49 pairs of extracted maxillary right and left human first premolars. The chemical composition of the labial enamel surface of all 49 pairs of teeth was determined using a non-destructive technique known as XPS. Since no significant difference ($p>0.05$) in chemical composition was found between the right and left sides of the teeth, they were randomly allocated into two independent studies. One study examined the relationship between chemical composition and *in vitro* bond strength, the other focused on the relationship between composition and qualitative acid etching pattern as observed using SEM. The inter-relationship between qualitative etching pattern and bond strength was determined indirectly using regression analysis with chemical composition serving as a common denominator as the composition of the left and right sides was not significantly different.

5.2 SIGNIFICANCE OF EACH STUDY

5.2.1 CHEMICAL COMPOSITION OF MAXILLARY RIGHT AND LEFT FIRST PREMOLAR

This study utilized XPS to determine the chemical composition of human maxillary right and left first premolars. The significance of using XPS for the determination of the chemical profile of the enamel surface is that it is a non-destructive method. The chemical composition of the samples can be determined and these samples allocated for further studies as they are not destroyed during XPS analysis.

In this study, since the enamel samples were not destroyed, we were able to further carry out two independent studies examining how chemical composition may affect *in vitro* bond strength and qualitative enamel etching pattern. As there was no significant difference (p =0.457) detected in the chemical profile of right and left sides of the teeth, the chemical composition of enamel surface can serve as a link to establish the relationship between etching pattern and *in vitro* bond strength.

5.2.2 CHEMICAL COMPOSITION OF ENAMEL SURFACE AS A PREDICTOR OF *IN VITRO* SHEAR BOND STRENGTH

This study utilized XPS to determine the chemical composition of enamel and MTS machine to determine the *in vitro* shear bond strength. Multiple linear regression models were generated and did not find any of the ions to be a significant predictor of *in vitro* bond strength. With a relatively large sample size of 49 enamel samples and a post hoc power analysis of 80%, there is sufficient power to report a negative finding.

Since a lack of relationship between enamel chemical composition and *in vitro* bond strength was found in this study (p =0.170), other factors must play an important role in the variation of bond strength frequently found in laboratory study. An interesting observation was the large variation in convexity of the buccal enamel surfaces after they have been sectioned off from the remaining tooth. It is possible that when looking at an entire tooth, there were too many distracters which may mask the variation in the shape of the buccal enamel surface. However, when the buccal surface was sectioned and viewed in isolation, the variation became easier to notice.

It is believed that this variation in the convexity of enamel surface may result in a poor adaptation of the bracket base and contribute to erratic bond strength[1]. It is recommended that future research examine the shape of enamel as a potential source contributing to the large variances found in laboratory bonding study. It would also be important to quantify how much of the variation in shape is needed to have an effect on the bond strength. Given the technologies that are available, this is a goal that can be achieved.

5.2.3 THE EFECT OF CHEMICAL COMPOSITION ON THE QUALITATIVE ETCHING PATTERN AND THE INTER-RELATIONSHIP BETWEEN ETHICNG PATTERN AND *IN VITRO* BOND STRENGTH

This study utilized XPS to determine the enamel chemical composition and SEM to classify the qualitative etching pattern of acid-etched enamel surface. There was no significant relationship ($p = 0.875$) between chemical composition and etching pattern and no significant relationship ($p = 0.832$) between etching pattern and *in vitro* bond strength.

It is believed that that current over-simplistic qualitative enamel etching classification may be responsible for the lack of relationship between the studied variables. Under the existing etching classification, the etching pattern that occupied the majority of the etched surface is considered to constitute the etching pattern of the enamel surface. That is, an enamel with a 1% poor etch and a 99% ideal etch would be classified as Type 1 pattern. At the same time, an etched enamel surface with a 25% poor etch and a 75% ideal etch would also identified as Type 1 pattern. These two etched enamel surfaces have different etch characteristics, however under the current qualitative etching classification system, they are categorized into the same etch pattern.

Further, Brannstrom[2] suggested that SEM is a poor intermediate tool for establishing the relationship between etch pattern and *in vitro* bond strength as the electron beam used in SEM cannot reach into the deep channels, depressions and the irregularities of the etched enamel surface which Brannstrom considered as more important in predicting the *in vitro* bond strength. In essence, Brannstrom[2] questioned the validity of SEM as a tool for studying *in vitro* bond strength. Instead, the use of a quantitative technique is required for the characterization of the etched enamel surface.

The development of a quantitative method for measuring the surface irregularities of the etched enamel surface has recently been undertaken by Orellana *et al.*[3]. The study utilized argon gas in a pressurized environment to coat the micro-porosities created by the acid etching process. The total surface area of the micro-porosities can thus be determined using this method.

It is believed with further refinement and development of this quantitative method of determining the etched surface area, our understanding of the relationship between chemical composition, acid etching and *in vitro* bond strength can be elucidated.

5.3 LIMITATIONS ASSOCIATED WITH THE STUDY

The relationship between acid etch pattern and *in vitro* bond strength was conducted using equivalent teeth. Because the two properties were not determined using the same teeth as SEM analysis effectively destroyed the ability to conduct *in vitro* bond study due to gold sputtering, the outcomes should be treated with some caution, despite the fact that the statistical method of regression analysis is commonly used to investigate and model the relationship between a response and one or more explanatory variables which may be unconnected. The use of regression model allows data from different sources to be examined for a possible relationship, but the results should be interpreted with caution.

The second limitation of the current study is that it is an *in vitro* study. Even though the MTS machine is considered the gold standard when it comes to assessing bond strength values, the results are nevertheless, obtained *in vitro*. It would be preferable to record *in vivo* measurements to assess bond strength, since the bracket bonding systems being tested are intended to be utilized *in vivo* and not in an *in vitro* environment.[4].

It has been shown that the results obtained from *in vitro* studies do not always correlate well with those achieved *in vivo*[5]. Clinical studies would be required to substantiate laboratory experiments. Therefore, it is important to follow the actual clinical bond failure rate. This research is designed so the *in vivo* bond failure rate can be tracked and the data correlated to the *in vitro* study in the future.

5.4 RECOMMENDATIONS ON FUTURE RESEARCH

The results of the current study suggested that a quantitative method of characterizing the etched enamel surface is needed to supplement or replace the existing over-simplistic qualitative enamel acid etching pattern. Efforts for the development of a quantitative method has been undertaken by Orellana *et al.*[3] The development of a quantitative technique provides the potential to enhance our understanding of the relationship between the amount of enamel surface area etched out by an acid etchant and the *in vitro* bond strength. It would also be important to determine the minimal amount of enamel surface area required to be etched out for adequate bond strength so the irreversible enamel loss due to acid etching can be minimized.

A second area of future study identified as a result of the current study is the need to study the effect of variation in the shape of the buccal enamel surface on *in vitro* bond strength. While it is recognized that variation in the convexity of enamel surface may result in a poor adaptation to the base of the brackets[1] it is not clear how much of the variation is needed to have an effect on the bond strength. With the technology available, it may be possible to use laser to scan the buccal surface of the enamel surface and bracket base and determine the closeness of the fit using custom-made computer software. This will allow quantification of the differences between enamel surface and bracket base which may be an important predictor of the large variances frequently noted in laboratory bonding studies.

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Appendices

Appendix A: Health Research Ethics Approval

Health Research Ethics Board

213 Heritage Medical Research Centre
University of Alberta, Edmonton, Alberta T6G 2S2
p.780.492.9724 (Biomedical Panel)
p.780.492.0302 (Health Panel)
p.780.492.0459
p.780.492.0839
f.780.492.7808

ETHICS APPROVAL FORM

Date: **August 2005**

Name(s) of Principal Investigator(s): **Dr. Paul Major**

Department: **Dentistry**

Title: **Surface characteristics of extracted human maxillary premolar enamel as a predictor of orthodontic bond strength: An in vivo and in vitro study**

The Health Research Ethics Board (Biomedical Panel) has reviewed the protocol involved in this project which has been found to be acceptable within the limitations of human experimentation.

Specific Comments:

The Research Ethics Board assessed all matters required by section 50(1)(a) of the Health Information Act. Subject consent for access to identifiable health information is required for the research described in the ethics application, and appropriate procedures for such consent have been approved by the REB Panel. The REB has also reviewed and approved the patient information material and consent form.

for J Bamforth
D. W. Morrish, M.D.
Chairman, Health Research Ethics Board
Biomedical Panel

AUG 2 6 2005
Date of Approval Release

This approval is valid for one year

Issue #5968

UNIVERSITY OF ALBERTA
Capital Health
CARITAS HEALTH GROUP

Appendix B: Patient / Parent consent form

PARENT CONSENT FORM

Title of Project: Surface characteristics of extracted human maxillary premolar enamel as a predictor of orthodontic bond strength: An in vivo and in intro study

Principal Investigators: Dr. Leo Lou; Dr. Paul Major

Contact Number: 780-492-4469

Department of Dentistry at the University of Alberta

Co-Investigators: Dr. Gieson Heo; Contact Number: 780-492-4469
Department of Dentistry at the University of Alberta
Dr. Alan Nelson Contact Number: 780-492-7380
Department of Chemical and Material Engineering at the University of Alberta

Part 2 (to be completed by the research subject):

Yes **No**

Do you understand that your child has been asked to participate in a research study?

Have you read and received a copy of the attached Information Sheet?

Do you understand the benefits and risks involved in taking part in this research study?

Have you had an opportunity to ask questions and discuss this study?

Do you understand that you are free to withdraw your child from the study at any time, without having to give a reason and without affecting your child's future medical care?

Do you understand who will have access to your child's records, including personally identifiable health information?

Do you want the investigator(s) to inform your child's family doctor or paediatrician? that your child is participating in this research study? Doctor's name ________________

Who explained this study to you? ______________________

Child's Name ______________________

I agree for my child to take part in this study: YES ☐ NO ☐

Signature of Parent or Guardian ______________________
Date & Time ________________

(Printed Name) ______________________

Signature of Witness ______________________
Date & Time ________________

Signature of Investigator or Designee ______________________
Date & Time ________________

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH SUBJECT

Appendix C: Patient recruitment letter

Research Information for participant and participant's parents/guardians

Research Title
Surface characteristics of extracted human maxillary premolar enamel as a predictor of orthodontic bond strength: An in vivo and in vitro study

Principal Investigators
Dr. Leo Lou (Department of Dentistry, 780-492-4469)
Dr. Paul Major (Department of Dentistry, 780-492-4469)

Co-Investigators
Dr. Alan Nelson (Department of Chemical and Material Engineering, 780-492-7380)
Dr. Giseon Heo (Department of Dentistry, 780-492-4469)

Purpose of the study
Treatment of braces involves attaching brackets onto teeth, but sometimes the brackets fail to stay attached to the teeth. If brackets come off the teeth, treatment time can be longer. The purpose of the study is to identify which characteristics of the surface of teeth make brackets stay on or come off the teeth.

Eligibility
If you need to have upper premolar teeth removed as part of your treatment with braces, you are invited to participate in the study.

Procedure
Your teeth will be removed in the Department of Dentistry, at the University of Alberta by a dental specialist. Instead of throwing away the teeth which would normally be the case, we will keep them to study the surface characteristics. Only the enamel surface will be used in the study, the rest of the tooth will be discarded. Your teeth sample will not have your name and the teeth will be destroyed on completion of the study. Throughout your treatment with braces, we will also access your treatment chart to keep track of how many brackets come off from your teeth.

Benefits/risks
Since the study is on teeth that are normally thrown away, your treatment with braces will not be affected in any way and there will be no additional risks other than routine risks and discomforts associated with regular treatment with braces and removal of teeth. By participating in the study, you will help us understand why brackets stay on so well in one person but come off easily on another person.

Confidentiality
The information obtained from your chart and as a result of your participation in this study will be coded and will not contain any identifiable data. Only the study investigators will have access to the data. It may be reviewed by the Health Research Ethics Board if necessary but your name will never be revealed. This information will be kept in a locked filing cabinet for at lest five years after the study is completed.

Voluntary participation
Participation in this study is voluntary. You may withdraw from the study at any time. Non-participation will not affect your treatment with braces. Your permission is being requested to use sample of enamel (surface of tooth) from your extracted teeth.

Contact person
If you have any questions about any aspect of this study, you may contact Dr. Kenneth Zakariasen, Chairperson of the Department of Dentistry, at 780-492-3312. You can also contact Dr. Leo Lou and Dr. Paul Major at 780-492-4469 for more information about this study.

Appendix D: Sample size calculation

Sample size calculation for the study is based on the formula for multiple regression of $N = (L/f^2) + K + 1$, where $f^2 = R^2/1\text{-}R^2$

R^2 = % of variation in outcome variable explained by explanatory variables

$f^2 = 0.2/\ 1\text{-}0.2 = 0.25$

K = number of independent variables = 5

$L_{k5,\,\alpha=0.05} = 12.83$ from statistical table

$$N = 12.83 / 0.25 + 5 + 1 = 57.32 = 58$$

A minimum of 58 maxillary teeth is required for each of SEM and XPS analyses. That is, a minimal total of 116 teeth (58x2) are needed.

L Values for α = .05

	Power										
k_B	.10	.30	.50	.60	.70	.75	.80	.85	.90	.95	.99
1	.43	2.06	3.84	4.90	6.17	6.94	7.85	8.98	10.51	13.00	18.37
2	.62	2.78	4.96	6.21	7.76	8.59	9.64	10.92	12.65	15.44	21.40
3	.78	3.30	5.76	7.15	8.79	9.77	10.90	12.30	14.17	17.17	23.52
4	.91	3.74	6.42	7.92	9.68	10.72	11.94	13.42	15.41	18.57	25.24
5	1.03	4.12	6.95	8.59	10.45	11.55	12.83	14.39	16.47	19.76	26.73
6	1.13	4.46	7.50	9.19	11.14	12.29	13.62	15.26	17.42	20.86	28.05
7	1.22	4.77	7.97	9.73	11.77	12.96	14.35	16.04	18.28	21.84	29.25
8	1.32	5.06	8.41	10.24	12.35	13.59	15.02	16.77	19.08	22.74	30.36
9	1.40	5.33	8.82	10.71	12.89	14.17	15.65	17.45	19.83	23.59	31.39
10	1.49	5.59	9.19	11.15	13.40	14.72	16.24	18.09	20.53	24.39	32.37
11	1.56	5.83	9.56	11.58	13.89	15.24	16.80	18.70	21.20	25.14	33.29
12	1.64	6.06	9.90	11.98	14.35	15.74	17.34	19.28	21.83	25.86	34.16
13	1.71	6.29	10.24	12.36	14.80	16.21	17.85	19.83	22.44	26.55	35.00
14	1.78	6.50	10.55	12.73	15.22	16.67	18.34	20.36	23.02	27.20	35.81
15	1.84	6.71	10.86	13.09	15.63	17.11	18.81	20.87	23.58	27.84	36.58
16	1.90	6.91	11.16	13.43	16.03	17.53	19.27	21.37	24.13	28.45	37.33
18	2.03	7.29	11.73	14.09	16.78	18.34	20.14	22.31	25.16	29.62	38.76
20	2.14	7.65	12.26	14.71	17.50	19.11	20.96	23.20	26.13	30.72	40.10
22	2.25	8.00	12.77	15.30	18.17	19.83	21.74	24.04	27.06	31.77	41.37
24	2.36	8.33	13.02	15.87	18.82	20.53	22.49	24.85	27.94	32.76	42.59
28	2.56	8.94	14.17	16.93	20.04	21.83	23.89	26.36	29.60	34.64	44.87
32	2.74	9.52	15.02	17.91	21.17	23.04	25.19	27.77	31.14	36.37	46.98
36	2.91	10.06	15.82	18.84	22.23	24.18	26.41	29.09	32.58	38.00	48.96
40	3.08	10.57	16.56	19.71	23.23	25.25	27.56	30.33	33.94	39.54	50.83
50	3.46	11.75	18.31	21.72	25.53	27.71	30.20	33.19	37.07	43.07	55.12
60	3.80	12.81	19.88	23.53	27.61	29.94	32.59	35.77	39.89	46.25	58.98
70	4.12	13.79	21.32	25.20	29.52	31.98	34.79	38.14	42.48	49.17	62.53
80	4.41	14.70	22.67	26.75	31.29	33.88	36.83	40.35	44.89	51.89	65.83
90	4.69	15.56	23.93	28.21	32.96	35.67	38.75	42.14	47.16	54.44	68.93
100	4.95	16.37	25.12	29.59	34.54	37.36	40.56	44.37	49.29	56.85	71.64

Appendix E: Raw data for the elements detected by XPS, measured in atomic concentration percent

Sample	Location	Na	Zn	O	N	Ca	C	P	Si	Cl	S	F	Mg	CO_3	$C0_3$/P
AB11	14	0.39	0.30	31.50	7.07	5.28	49.98	4.01	0.72	0.00	0.00	0.37	0.38	2.50	0.5
AH8	14	0.28	0.14	32.86	6.51	6.52	47.44	5.08	0.23	0.00	0.43	0.51	0.00	4.41	0.76
AH9	14	0.42	0.25	37.88	5.14	7.95	40.89	5.50	1.01	0.00	0.00	0.57	0.39	1.77	0.43
BE61	14	0.31	0.27	28.98	7.43	3.04	56.84	2.31	0.61	0.00	0.20	0.00	0.00	5.77	2.28
BE63	14	0.29	0.10	37.98	5.14	10.04	38.46	6.74	0.59	0.00	0.29	0.38	0.00	1.20	0.16
BR54	14	0.35	0.05	26.81	8.46	3.05	57.60	2.36	0.63	0.00	0.45	0.24	0.00	1.21	0.33
CH71	14	0.30	0.00	31.16	6.85	3.76	54.25	2.64	0.42	0.00	0.47	0.16	0.00	1.37	0.44
CH74	14	0.42	0.08	27.91	8.61	2.38	58.23	1.72	0.63	0.02	0.01	0.00	0.00	7.15	2.69
DA32	14	0.41	0.23	34.80	4.89	4.44	50.58	3.11	1.49	0.01	0.04	0.00	0.00	2.81	0.71
DE43	14	0.44	0.04	33.44	5.95	5.19	49.55	3.89	0.93	0.00	0.34	0.24	0.00	2.50	0.55
DH6	14	0.49	0.16	37.89	4.37	9.55	39.01	6.80	0.68	0.00	0.71	0.33	0.00	0.71	0.09
DO35	14	0.25	0.16	29.49	8.82	5.16	52.23	3.49	0.40	0.00	0.00	0.00	0.00	2.37	0.57
EL11	14	0.19	0.10	26.62	9.66	4.42	55.11	2.96	0.51	0.00	0.29	0.14	0.00	3.35	1.13
EM6	14	0.33	0.20	31.63	7.38	4.82	51.53	3.39	0.70	0.01	0.02	0.00	0.00	6.97	1.54
FA91	14	0.34	0.06	34.59	5.00	7.47	45.47	5.34	0.78	0.00	0.59	0.36	0.00	2.16	0.31
GE15	14	0.14	0.28	27.47	8.01	2.98	57.87	1.92	1.00	0.00	0.24	0.10	0.00	4.91	1.95
HA88	14	0.70	0.00	26.86	10.27	4.74	53.00	3.63	0.25	0.55	0.00	0.00	0.00	3.97	0.9
HE44	14	0.18	0.24	26.54	7.25	1.70	61.94	1.43	0.26	0.00	0.21	0.25	0.00	4.25	2.55
HO48	14	0.33	0.00	27.22	10.34	4.28	54.83	2.99	0.00	0.00	0.00	0.00	0.00	4.88	1.16
HU52	14	0.17	0.28	33.67	5.91	7.70	46.18	4.87	0.46	0.00	0.37	0.40	0.00	2.59	0.4
HU54	14	0.16	0.58	24.46	8.50	4.13	58.46	2.99	0.70	0.00	0.00	0.00	0.00	3.01	0.91
KH15	14	0.29	0.02	36.74	4.72	8.34	42.51	6.12	0.40	0.00	0.39	0.48	0.00	0.56	0.08
MA119	14	0.25	0.14	33.25	6.56	5.31	50.26	3.64	0.51	0.02	0.05	0.00	0.00	0.00	0
MA122	14	0.38	0.28	33.22	7.61	7.40	45.36	5.34	0.41	0.00	0.00	0.00	0.00	2.24	0.36
MO55	14	0.45	0.17	34.35	5.56	7.40	45.54	5.21	0.43	0.00	0.55	0.34	0.00	3.48	0.55
MO57	14	0.44	0.11	39.77	3.65	9.17	38.18	6.59	0.59	0.00	1.11	0.40	0.00	2.00	0.26
MU35	14	1.89	0.00	23.90	8.33	4.57	55.41	3.20	0.54	2.16	0.00	0.00	0.00	2.84	0.71
NG14	14	0.27	0.14	29.31	6.96	3.38	56.39	2.35	0.54	0.00	0.36	0.32	0.00	4.83	1.74
NU4	14	0.37	0.00	30.70	6.45	2.56	56.82	1.93	0.70	0.00	0.36	0.09	0.00	2.46	1.1

PA62	14	0.50	0.38	39.51	4.17	9.47	37.51	6.83	0.78	0.00	0.33	0.52	0.00	2.26	0.28
PA66	14	1.12	0.27	32.54	6.09	5.86	47.97	4.48	0.98	0.00	0.00	0.34	0.36	3.64	0.66
PE45	14	0.09	0.06	22.26	10.23	2.15	63.11	1.34	0.45	0.00	0.15	0.15	0.00	5.23	3.17
RE35	14	0.36	0.39	36.70	4.30	8.59	42.53	5.93	0.40	0.00	0.43	0.37	0.00	1.87	0.28
RE46	14	0.28	0.00	29.38	7.62	5.18	53.22	3.74	0.58	0.00	0.00	0.00	0.00	2.98	0.68
RE50	14	0.64	0.09	35.58	5.67	6.66	44.50	4.91	1.08	0.00	0.63	0.24	0.00	2.00	0.37
RI19	14	0.31	0.24	27.81	7.90	4.67	54.56	3.38	0.50	0.00	0.00	0.42	0.20	3.97	1.09
RO62	14	0.46	0.31	39.98	3.10	11.26	36.52	7.19	0.21	0.00	0.50	0.47	0.00	1.20	0.17
SI34	14	0.46	0.07	38.14	4.35	8.10	41.39	5.61	0.92	0.00	0.50	0.46	0.00	0.63	0.09
SM17	14	0.30	0.16	35.23	5.11	8.87	44.03	5.31	0.39	0.00	0.20	0.41	0.00	2.41	0.34
SM19	14	0.22	0.11	29.65	6.83	3.87	55.44	2.76	0.40	0.00	0.52	0.21	0.00	3.22	0.9
TA17	14	0.30	0.30	30.61	8.50	5.68	50.03	3.91	0.66	0.00	0.00	0.00	0.00	2.63	0.54
TI11	14	0.21	0.00	27.14	8.48	4.66	55.68	3.41	0.41	0.00	0.00	0.00	0.00	2.51	0.74
TO11	14	0.06	0.11	23.75	6.67	3.93	61.98	2.68	0.38	0.00	0.24	0.20	0.00	2.20	0.62
TU15	14	0.27	0.23	34.19	5.73	5.91	48.90	3.98	0.52	0.00	0.25	0.00	0.00	5.90	1.23
WE17	14	0.24	0.11	30.19	6.58	4.45	54.34	3.27	0.31	0.00	0.33	0.19	0.00	4.52	1.23
WE24	14	0.38	0.10	34.38	7.69	7.94	43.55	4.67	0.61	0.00	0.35	0.34	0.00	2.48	0.42
WI48	14	0.20	0.00	31.27	8.45	6.24	49.11	4.40	0.33	0.00	0.00	0.00	0.00	2.06	0.41
YU5	14	0.15	0.00	27.10	8.30	5.42	54.60	3.95	0.46	0.00	0.00	0.00	0.00	2.60	0.53
ZH4	14	0.32	0.13	30.40	6.77	6.63	50.14	4.45	0.50	0.00	0.44	0.23	0.00	2.79	0.48
AB11	24	0.33	0.37	39.40	3.87	9.83	37.60	6.92	0.77	0.00	0.00	0.59	0.32	1.58	0.2
AH8	24	0.21	0.25	28.09	5.48	5.92	54.28	4.46	0.66	0.00	0.30	0.36	0.00	3.01	0.57
AH9	24	0.66	0.13	37.02	6.05	6.91	42.34	5.08	1.11	0.00	0.00	0.54	0.16	1.15	0.17
BE61	24	0.31	0.06	33.48	6.68	5.38	49.78	3.64	0.52	0.02	0.13	0.00	0.00	4.15	0.89
BE63	24	0.70	0.44	35.80	5.48	5.62	45.29	4.48	2.10	0.00	0.07	0.00	0.00	4.70	0.95
BR54	24	0.53	0.04	24.98	8.27	1.37	62.79	1.18	0.49	0.00	0.34	0.05	0.00	2.20	1.5
CH71	24	0.18	0.00	35.54	4.57	7.96	44.14	5.63	1.27	0.00	0.25	0.45	0.00	1.44	0.21
CH74	24	0.18	0.24	28.42	8.06	2.79	57.49	1.92	0.65	0.02	0.23	0.00	0.00	11.11	5.24
DA32	24	0.43	0.37	36.59	4.16	8.21	43.33	5.82	0.82	0.01	0.25	0.00	0.00	2.55	0.37
DE43	24	0.49	0.09	34.05	4.57	5.31	49.83	3.99	1.20	0.00	0.31	0.16	0.00	1.60	0.33
DH6	24	0.93	0.05	41.99	4.03	9.89	34.73	7.12	0.45	0.00	0.49	0.33	0.00	0.50	0.06
DO35	24	0.44	0.12	28.95	9.67	4.21	53.09	3.08	0.45	0.00	0.00	0.00	0.00	3.33	0.93

EL11	24	1.04	0.11	28.41	8.60	5.26	52.39	3.26	0.37	0.00	0.39	0.16	0.00	4.87	0.01
EM6	24	0.46	0.26	33.68	5.96	5.90	48.18	4.27	1.09	0.02	0.17	0.00	0.00	6.43	1.24
FA91	24	0.36	0.14	35.96	4.75	7.11	44.62	5.08	1.05	0.00	0.60	0.34	0.00	0.00	0
GE15	24	0.29	0.22	23.93	10.68	1.91	61.14	1.17	0.39	0.00	0.19	0.08	0.00	4.44	2.85
HA88	24	0.15	0.00	24.83	8.49	4.19	57.83	3.11	1.41	0.00	0.00	0.00	0.00	3.19	0.79
HE44	24	0.17	0.23	25.97	7.08	1.75	62.54	1.40	0.31	0.00	0.46	0.08	0.00	4.40	2.67
HO48	24	0.39	0.00	27.23	6.59	3.88	59.27	2.65	0.00	0.00	0.00	0.00	0.00	5.39	1.8
HU52	24	0.27	0.07	38.03	5.50	9.83	38.93	6.25	0.28	0.00	0.36	0.36	0.00	1.24	0.16
HU54	24	0.27	0.16	25.92	9.09	4.48	56.46	3.23	0.39	0.00	0.00	0.00	0.00	3.32	0.9
KH15	24	0.34	0.08	34.44	4.70	4.92	50.82	3.76	0.60	0.00	0.21	0.12	0.00	2.33	0.56
MA119	24	0.33	0.26	29.43	7.55	3.43	55.94	2.22	0.79	0.02	0.02	0.00	0.00	4.20	1.58
MA122	24	0.36	0.00	29.82	10.16	5.78	49.38	4.34	0.17	0.00	0.00	0.00	0.00	2.88	0.66
MO55	24	0.31	0.14	34.45	5.84	6.61	46.46	4.88	0.37	0.00	0.65	0.30	0.00	2.98	0.49
MO57	24	0.67	0.26	44.38	2.82	11.17	30.66	8.27	0.45	0.00	0.79	0.53	0.00	1.13	0.12
MU35	24	1.29	0.00	23.60	8.13	3.43	59.15	2.49	0.65	1.26	0.00	0.00	0.00	3.33	1.2
NG14	24	0.49	0.17	28.58	6.84	2.83	57.55	2.31	0.84	0.00	0.17	0.21	0.00	5.44	1.97
NU4	24	0.52	0.00	31.29	8.15	3.24	53.11	2.62	0.48	0.00	0.17	0.42	0.00	2.62	0.9
PA62	24	0.18	0.27	32.87	5.60	5.54	50.69	3.73	0.52	0.00	0.43	0.19	0.00	4.31	0.54
PA66	24	0.60	0.25	35.83	5.86	7.17	43.93	4.95	0.74	0.00	0.00	0.39	0.28	2.77	0.53
PE45	24	0.22	0.18	34.36	5.36	8.02	44.99	5.51	0.68	0.00	0.27	0.42	0.00	2.11	0.32
RE35	24	0.50	0.30	36.00	5.02	6.71	44.96	5.04	0.49	0.00	0.55	0.44	0.00	2.69	0.52
RE46	24	0.27	0.00	24.00	9.37	2.48	61.41	1.69	0.59	0.19	0.00	0.00	0.00	2.97	1.35
RE50	24	0.36	0.02	33.44	5.89	4.93	50.97	3.41	0.73	0.00	0.08	0.18	0.00	2.44	0.62
RI19	24	0.20	0.11	30.44	5.75	3.28	57.00	2.49	0.41	0.00	0.00	0.22	0.09	3.47	1.75
RO62	24	0.88	0.23	32.88	3.68	8.55	46.83	5.89	0.44	0.00	0.51	0.11	0.00	1.16	0.16
SI34	24	0.30	0.16	37.27	5.31	7.44	42.43	5.46	0.79	0.00	0.50	0.34	0.00	2.62	0.4
SM17	24	0.20	0.30	30.49	6.58	6.81	49.57	4.89	0.51	0.00	0.40	0.24	0.00	2.25	0.38
SM19	24	0.28	0.08	30.30	6.39	5.72	51.46	4.01	0.99	0.00	0.51	0.27	0.00	0.27	0.06
TA17	24	0.43	0.06	30.53	9.84	5.10	49.94	3.78	0.32	0.00	0.00	0.00	0.00	3.16	0.73
TI11	24	0.45	0.08	23.64	6.23	3.64	63.16	2.13	0.66	0.00	0.00	0.00	0.00	2.20	0.91
TO11	24	0.32	0.02	25.34	7.31	3.30	60.80	2.16	0.33	0.00	0.18	0.24	0.00	2.73	1
TU15	24	0.30	0.21	34.19	6.03	6.37	48.08	4.21	0.48	0.00	0.13	0.00	0.00	5.76	1.15

WE17	24	0.18	0.12	28.19	7.66	2.91	57.08	2.51	0.35	0.00	0.53	0.47	0.00	3.29	1.5
WE24	24	0.27	0.24	28.86	8.95	5.35	51.30	3.63	0.71	0.00	0.48	0.20	0.00	2.48	0.59
WI48	24	0.45	0.00	24.65	10.76	3.12	58.75	2.16	0.12	0.00	0.00	0.00	0.00	3.01	1.11
YU5	24	0.59	0.00	27.16	8.57	5.42	52.87	4.24	0.63	0.52	0.00	0.00	0.00	2.62	0.51
ZH4	24	0.14	0.15	23.39	6.61	4.20	61.13	3.05	0.81	0.00	0.35	0.18	0.00	2.05	0.49

Appendix F: Raw data for difference in atomic concentration percent between teeth 14 and 24 (14 subtract 24) as measured using XPS (diff = difference between the left and right side of the teeth)

Sample	diffNa	diffZn	diffO	diffN	diffCa	diffC	diffP	diffSi	diffCl	diffS	diffF	diffMg	diffCO_3	diffCO_3/P
AB11	0.06	-0.07	-7.9	3.2	-4.55	12.4	-2.91	-0.05	0	0	-0.22	0.06	0.92	0.3
AH8	0.07	-0.11	4.77	1.03	0.6	-6.84	0.62	-0.43	0	0.13	0.15	0	1.4	0.19
AH9	-0.24	0.12	0.86	-0.91	1.04	-1.45	0.42	-0.1	0	0	0.03	0.23	0.62	0.26
BE61	0	0.21	-4.5	0.75	-2.34	7.06	-1.33	0.09	-0	0.07	0	0	1.62	1.39
BE63	-0.41	-0.34	2.18	-0.34	4.42	-6.83	2.26	-1.51	0	0.22	0.38	0	-3.5	-0.79
BR54	-0.18	0.01	1.83	0.19	1.68	-5.19	1.18	0.14	0	0.11	0.19	0	-0.99	-1.17
CH71	0.12	0	-4.38	2.28	-4.2	10.1	-2.99	-0.85	0	0.22	-0.29	0	-0.07	0.23
CH74	0.24	-0.16	-0.51	0.55	-0.41	0.74	-0.2	-0.02	0	-0.22	0	0	-3.96	-2.55
DA32	-0.02	-0.14	-1.79	0.73	-3.77	7.25	-2.71	0.67	0	-0.21	0	0	0.26	0.34
DE43	-0.05	-0.05	-0.61	1.38	-0.12	-0.28	-0.1	-0.27	0	0.03	0.08	0	0.9	0.22
DH6	-0.44	0.11	-4.1	0.34	-0.34	4.28	-0.32	0.23	0	0.22	0	0	0.21	0.03
DO35	-0.19	0.04	0.54	-0.85	0.95	-0.86	0.41	-0.05	0	0	0	0	-0.96	-0.36
EL11	-0.85	-0.01	-1.79	1.06	-0.84	2.72	-0.3	0.14	0	-0.1	-0.02	0	-1.52	1.12
EM6	-0.13	-0.06	-2.05	1.42	-1.08	3.35	-0.88	-0.39	-0	-0.15	0	0	0.54	0.3
FA91	-0.02	-0.08	-1.37	0.25	0.36	0.85	0.26	-0.27	0	-0.01	0.02	0	2.16	0.31
GE15	-0.15	0.06	3.54	-2.67	1.07	-3.27	0.75	0.61	0	0.05	0.02	0	0.47	-0.9
HA88	0.55	0	2.03	1.78	0.55	-4.83	0.52	-1.16	0.55	0	0	0	0.78	0.11
HE44	0.01	0.01	0.57	0.17	-0.05	-0.6	0.03	-0.05	0	-0.25	0.17	0	-0.15	-0.12
HO48	-0.06	0	-0.01	3.75	0.4	-4.44	0.34	0	0	0	0	0	-0.51	-0.64
HU52	-0.1	0.21	-4.36	0.41	-2.13	7.25	-1.38	0.18	0	0.01	0.04	0	1.35	0.24
HU54	-0.11	0.42	-1.46	-0.59	-0.35	2	-0.24	0.31	0	0	0	0	-0.31	0.01
KH15	-0.05	-0.06	2.3	0.02	3.42	-8.31	2.36	-0.2	0	0.18	0.36	0	-1.77	-0.48
MA119	-0.08	-0.12	3.82	-0.99	1.88	-5.68	1.42	-0.28	0	0.03	0	0	-4.2	-1.58
MA122	0.02	0.28	3.4	-2.55	1.62	-4.02	1	0.24	0	0	0	0	-0.64	-0.3
MO55	0.14	0.03	-0.1	-0.28	0.79	-0.92	0.33	0.06	0	-0.1	0.04	0	0.5	0.06
MO57	-0.23	-0.15	-4.61	0.83	-2	7.52	-1.68	0.14	0	0.32	-0.13	0	0.87	0.14
MU35	0.6	0	0.3	0.2	1.14	-3.74	0.71	-0.11	0.9	0	0	0	-0.49	-0.49
NG14	-0.22	-0.03	0.73	0.12	0.55	-1.16	0.04	-0.3	0	0.19	0.11	0	-0.61	-0.23

NU4	-0.15	0	-0.59	-1.7	-0.68	3.71	-0.69	0.22	0	0.19	-0.33	0	-0.16	0.2
PA62	0.32	0.11	6.64	-1.43	3.93	-13.2	3.1	0.26	0	-0.1	0.33	0	-2.05	-0.26
PA66	0.52	0.02	-3.29	0.23	-1.31	4.04	-0.47	0.24	0	0	-0.05	0.08	0.87	0.13
PE45	-0.13	-0.12	-12.1	4.87	-5.87	18.1	-4.17	-0.23	0	-0.12	-0.27	0	3.12	2.85
RE35	-0.14	0.09	0.7	-0.72	1.88	-2.43	0.89	-0.09	0	-0.12	-0.07	0	-0.82	-0.24
RE46	0.01	0	5.38	-1.75	2.7	-8.19	2.05	-0.01	-0.2	0	0	0	0.01	-0.67
RE50	0.28	0.07	2.14	-0.22	1.73	-6.47	1.5	0.35	0	0.55	0.06	0	-0.44	-0.25
RI19	0.11	0.13	-2.63	2.15	1.39	-2.44	0.89	0.09	0	0	0.2	0.11	0.5	-0.66
RO62	-0.42	0.08	7.1	-0.58	2.71	-10.3	1.3	-0.23	0	-0.01	0.36	0	0.04	0.01
SI34	0.16	-0.09	0.87	-0.96	0.66	-1.04	0.15	0.13	0	0	0.12	0	-1.99	-0.31
SM17	0.1	-0.14	4.74	-1.47	2.06	-5.54	0.42	-0.12	0	-0.2	0.17	0	0.16	-0.04
SM19	-0.06	0.03	-0.65	0.44	-1.85	3.98	-1.25	-0.59	0	0.01	-0.06	0	2.95	0.84
TA17	-0.13	0.24	0.08	-1.34	0.58	0.09	0.13	0.34	0	0	0	0	-0.53	-0.19
TI11	-0.24	-0.08	3.5	2.25	1.02	-7.48	1.28	-0.25	0	0	0	0	0.31	-0.17
TO11	-0.26	0.09	-1.59	-0.64	0.63	1.18	0.52	0.05	0	0.06	-0.04	0	-0.53	-0.38
TU15	-0.03	0.02	0	-0.3	-0.46	0.82	-0.23	0.04	0	0.12	0	0	0.14	0.08
WE17	0.06	-0.01	2	-1.08	1.54	-2.74	0.76	-0.04	0	-0.2	-0.28	0	1.23	-0.27
WE24	0.11	-0.14	5.52	-1.26	2.59	-7.75	1.04	-0.1	0	-0.13	0.14	0	0	-0.17
WI48	-0.25	0	6.62	-2.31	3.12	-9.64	2.24	0.21	0	0	0	0	-0.95	-0.7
YU5	-0.44	0	-0.06	-0.27	0	1.73	-0.29	-0.17	-0.5	0	0	0	-0.02	0.02
ZH4	0.18	-0.02	7.01	0.16	2.43	-11	1.4	-0.31	0	0.09	0.05	0	0.74	-0.01

Appendix G: Statistics for Chapter 2

Paired t-test to evaluate if a statistical significant difference exists between the atomic concentration percent of the elements on the right (14) and left (24) sides of the maxillary teeth

Since there were 14 outcome variables, a Bonferroni correction was applied (α / 14 = 0.05 / 14) making the significance level as 0.00357. The table below indicated no statistical significant differences between any of the elements on tooth 14 and 24.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Na14 - Na24	-.04327	.26003	.03715	-.11795	.03142	-1.165	48	.250
Pair 2	Zn14 - Zn24	.00816	.12819	.01831	-.02866	.04498	.446	48	.658
Pair 3	O14 - O24	.38204	3.83742	.54820	-.72019	1.48428	.697	48	.489
Pair 4	N14 - N24	.10918	1.53815	.21974	-.33262	.55099	.497	48	.622
Pair 5	Ca14 - Ca24	.34878	2.13797	.30542	-.26532	.96287	1.142	48	.259
Pair 6	C14 - C24	-.96816	6.33024	.90432	-2.78642	.85009	-1.071	48	.290
Pair 7	P14 - P24	.16694	1.43096	.20442	-.24408	.57796	.817	48	.418
Pair 8	Si14 - Si24	-.07020	.38511	.05502	-.18082	.04041	-1.276	48	.208
Pair 9	S14 - S24	.01796	.14697	.02100	-.02426	.06017	.855	48	.397
Pair 10	F14 - F24	.02571	.15293	.02185	-.01821	.06964	1.177	48	.245
Pair 11	CO314 - CO324	-.09184	1.45364	.20766	-.50937	.32570	-.442	48	.660
Pair 12	C03/P14 - C03/P24	-.09286	.75397	.10771	-.30942	.12371	-.862	48	.393
Pair 13	Cl14 - Cl24	.01449	.17134	.02448	-.03473	.06371	.592	48	.557
Pair 14	Mg14 - Mg24	.00980	.03827	.00547	-.00120	.02079	1.792	48	.079

Appendix H: Randomization Table to allocate the total enamel samples into an *in vitro* bonding group and a qualitative etching pattern group

patients	teeth	treat			ran
1	left	S	S: SEM		0.100681
1	right	X	X: XPS		0.382
2	left	X			0.596484
2	right	S			0.899106
3	left	X			0.88461
3	right	S			0.958464
4	left	X			0.014496
4	right	S			0.407422
5	left	S			0.138585
5	right	X			0.863247
6	left	S			0.045473
6	right	X			0.245033
7	left	X			0.03238
7	right	S			0.164129
8	left	S			0.01709
8	right	X			0.219611
9	left	X			0.285043
9	right	S			0.343089
10	left	S			0.357372
10	right	X			0.553636
11	left	S			0.355602
11	right	X			0.371838
12	left	S			0.466018
12	right	X			0.910306
13	left	S			0.303903
13	right	X			0.42616
14	left	S			0.806665
14	right	X			0.975707
15	left	S			0.256264
15	right	X			0.991241
16	left	S			0.053438
16	right	X			0.951689
17	left	X			0.705039
17	right	S			0.816523
18	left	S			0.466323
18	right	X			0.972503
19	left	X			0.300211
19	right	S			0.750206
20	left	X			0.351482
20	right	S			0.775658
21	left	X			0.074343
21	right	S			0.198431
22	left	X			0.064058

22	right	S			0.358348
23	left	X			0.487045
23	right	S			0.511216
24	left	X			0.373455
24	right	S			0.9859
25	left	X			0.040712
25	right	S			0.23072
26	left	X			0.004975
26	right	S			0.926145
27	left	X			0.100314
27	right	S			0.256691
28	left	S			0.679647
28	right	X			0.775689
29	left	S			0.724326
29	right	X			0.809107
30	left	X			0.085055
30	right	S			0.132267
31	left	S			0.626514
31	right	X			0.756157
32	left	X			0.17365
32	right	S			0.404798
33	left	X			0.552324
33	right	S			0.711509
34	left	S			0.181158
34	right	X			0.555162
35	left	S			0.686941
35	right	X			0.970275
36	left	X			0.528794
36	right	S			0.796686
37	left	S			0.262215
37	right	X			0.805658
38	left	X			0.177953
38	right	S			0.866756
39	left	S			0.059511
39	right	X			0.114841
40	left	S			0.738395
40	right	X			0.761559
41	left	S			0.925596
41	right	X			0.986297
42	left	S			0.544969
42	right	X			0.903867
43	left	X			0.500778
43	right	S			0.674978
44	left	S			0.145787
44	right	X			0.489822
45	left	X			0.037965
45	right	S			0.796258
46	left	X			0.67156

46	right	S			0.731681
47	left	S			0.152226
47	right	X			0.584521
48	left	S			0.377819
48	right	X			0.892178
49	left	X			0.200476
49	right	S			0.205786
50	left	S			0.325144
50	right	X			0.333964
51	left	X			0.300211
51	right	S			0.802179

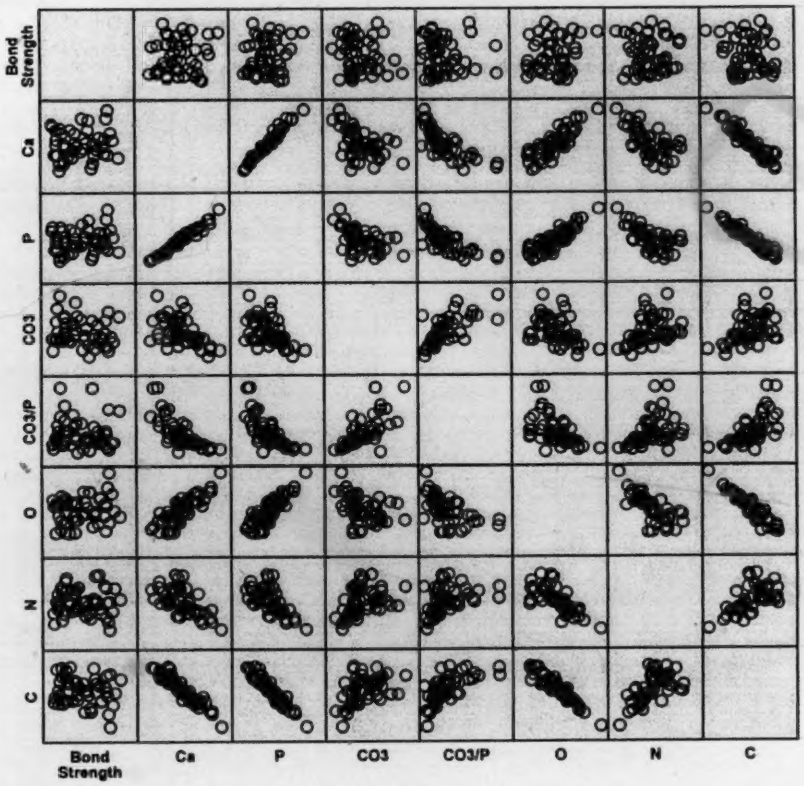
Appendix I: Raw data for in vitro shearing bond strength and atomic concentration percent for the detected elements in the enamel samples

Sample	BondStrength (MPa)	Na	Zn	O	N	Ca	C	P	Si	Cl	S	F	Mg	CO_3	CO_3/P
AB11-24B	10.60	0.33	0.37	39.40	3.87	9.83	37.60	6.92	0.77	0.00	0.00	0.59	0.32	1.58	0.2
AH8-14B	10.40	0.28	0.14	32.86	6.51	6.52	47.44	5.08	0.23	0.00	0.43	0.51	0.00	4.41	0.76
AH9-14B	8.00	0.42	0.25	37.88	5.14	7.95	40.89	5.50	1.01	0.00	0.00	0.57	0.39	1.77	0.43
BE61-24B	4.80	0.31	0.06	33.48	6.68	5.38	49.78	3.64	0.52	0.02	0.13	0.00	0.00	4.15	0.89
BE63-24B	4.60	0.70	0.44	35.80	5.48	5.62	45.29	4.48	2.10	0.00	0.07	0.00	0.00	4.7	0.95
BR54-24B	4.00	0.53	0.04	24.98	8.27	1.37	62.79	1.18	0.49	0.00	0.34	0.05	0.00	2.2	1.5
CH71-14B	8.90	0.30	0.00	31.16	6.85	3.76	54.25	2.64	0.42	0.00	0.47	0.16	0.00	1.37	0.44
CH74-14B	4.00	0.42	0.08	27.91	8.61	2.38	58.23	1.72	0.63	0.02	0.01	0.00	0.00	7.15	2.69
DA32-24B	4.10	0.43	0.37	36.59	4.16	8.21	43.33	5.82	0.82	0.01	0.25	0.00	0.00	2.55	0.37
DE43-24B	10.30	0.49	0.09	34.05	4.57	5.31	49.83	3.99	1.20	0.00	0.31	0.16	0.00	1.6	0.33
DH6-14B	8.10	0.49	0.16	37.89	4.37	9.55	39.01	6.80	0.68	0.00	0.71	0.33	0.00	0.5	0.09
DO35-14B	10.10	0.25	0.16	29.49	8.82	5.16	52.23	3.49	0.40	0.00	0.00	0.00	0.00	2.37	0.57
EL11-24B	6.40	1.04	0.11	28.41	8.60	5.26	52.39	3.26	0.37	0.00	0.39	0.16	0.00	4.87	0.01
EM6-24B	6.20	0.46	0.26	33.68	5.96	5.90	48.18	4.27	1.09	0.02	0.17	0.00	0.00	6.43	1.24
FA91-14B	4.40	0.34	0.06	34.59	5.00	7.47	45.47	5.34	0.78	0.00	0.59	0.36	0.00	2.16	0.31
GE15-14B	4.60	0.14	0.28	27.47	8.01	2.98	57.87	1.92	1.00	0.00	0.24	0.10	0.00	4.91	1.95
HA88-14B	9.30	0.70	0.00	26.86	10.27	4.74	53.00	3.63	0.25	0.55	0.00	0.00	0.00	3.97	0.9
HE44-24B	8.50	0.17	0.23	25.97	7.08	1.75	62.54	1.40	0.31	0.00	0.46	0.08	0.00	4.4	2.67
HO48-24B	10.80	0.39	0.00	27.23	6.59	3.88	59.27	2.65	0.00	0.00	0.00	0.00	0.00	5.39	1.8
HU52-24B	7.20	0.27	0.07	38.03	5.50	9.83	38.93	6.25	0.28	0.00	0.36	0.36	0.00	1.24	0.16
HU54-14B	10.30	0.16	0.58	24.46	8.50	4.13	58.46	2.99	0.70	0.00	0.00	0.00	0.00	3.01	0.91
KH15-24B	7.40	0.34	0.08	34.44	4.70	4.92	50.82	3.76	0.60	0.00	0.21	0.12	0.00	2.33	0.56
MA119-24B	5.80	0.33	0.26	29.43	7.55	3.43	55.94	2.22	0.79	0.02	0.02	0.00	0.00	4.2	1.58
MA122-24B	8.80	0.36	0.00	29.82	10.16	5.78	49.38	4.34	0.17	0.00	0.00	0.00	0.00	2.88	0.66
MO55-14B	6.20	0.45	0.17	34.35	5.56	7.40	45.54	5.21	0.43	0.00	0.55	0.34	0.00	3.48	0.55
MO57-24B	10.80	0.67	0.26	44.38	2.82	11.17	30.66	8.27	0.45	0.00	0.79	0.53	0.00	1.13	0.12
MU35-14B	6.00	1.89	0.00	23.90	8.33	4.57	55.41	3.20	0.54	2.16	0.00	0.00	0.00	2.84	0.71

NG14-14B	12.30	0.27	0.14	29.31	6.96	3.38	56.39	2.35	0.54	0.00	0.36	0.32	0.00	4.83	1.74
NU4-14B	8.70	0.37	0.00	30.70	6.45	2.56	56.82	1.93	0.70	0.00	0.36	0.09	0.00	2.46	1.1
PA62-24B	3.50	0.18	0.27	32.87	5.60	5.54	50.69	3.73	0.52	0.00	0.43	0.19	0.00	4.31	0.54
PA66-14B	7.70	1.12	0.27	32.54	6.09	5.86	47.97	4.48	0.98	0.00	0.00	0.34	0.36	3.64	0.66
PE45-24B	5.50	0.22	0.18	34.36	5.36	8.02	44.99	5.51	0.68	0.00	0.27	0.42	0.00	2.11	0.32
RE35-24B	9.60	0.50	0.30	36.00	5.02	6.71	44.96	5.04	0.49	0.00	0.55	0.44	0.00	2.69	0.52
RE46-24B	4.60	0.27	0.00	24.00	9.37	2.48	61.41	1.69	0.59	0.19	0.00	0.00	0.00	2.97	1.35
RE50-14B	9.50	0.64	0.09	35.58	5.67	6.66	44.50	4.91	1.08	0.00	0.63	0.24	0.00	2	0.37
RI19-14B	5.90	0.31	0.24	27.81	7.90	4.67	54.56	3.38	0.50	0.00	0.00	0.42	0.20	3.97	1.09
RO62-24B	3.00	0.88	0.23	32.88	3.68	8.55	46.83	5.89	0.44	0.00	0.51	0.11	0.00	1.16	0.16
SI34-24B	11.20	0.30	0.16	37.27	5.31	7.44	42.43	5.46	0.79	0.00	0.50	0.34	0.00	2.62	0.4
SM17-14B	2.90	0.30	0.16	35.23	5.11	8.87	44.03	5.31	0.39	0.00	0.20	0.41	0.00	2.41	0.34
SM19-14B	3.70	0.22	0.11	29.65	6.83	3.87	55.44	2.76	0.40	0.00	0.52	0.21	0.00	3.22	0.9
TA17-24B	4.70	0.43	0.06	30.53	9.84	5.10	49.94	3.78	0.32	0.00	0.00	0.00	0.00	3.16	0.73
TI11-24B	5.20	0.45	0.08	23.64	6.23	3.64	63.16	2.13	0.66	0.00	0.00	0.00	0.00	2.2	0.91
TO11-14B	3.30	0.06	0.11	23.75	6.67	3.93	61.98	2.68	0.38	0.00	0.24	0.20	0.00	2.2	0.62
TU15-24B	3.60	0.30	0.21	34.19	6.03	6.37	48.08	4.21	0.48	0.00	0.13	0.00	0.00	5.76	1.15
WE17-14B	7.00	0.24	0.11	30.19	6.58	4.45	54.34	3.27	0.31	0.00	0.33	0.19	0.00	4.52	1.23
WE24-14B	3.50	0.38	0.10	34.38	7.69	7.94	43.55	4.67	0.61	0.00	0.35	0.34	0.00	2.48	0.42
WI48-14B	11.50	0.20	0.00	31.27	8.45	6.24	49.11	4.40	0.33	0.00	0.00	0.00	0.00	2.06	0.41
YU5-14B	4.80	0.15	0.00	27.10	8.30	5.42	54.60	3.95	0.46	0.00	0.00	0.00	0.00	2.6	0.53
ZH4-14B	7.50	0.32	0.13	30.40	6.77	6.63	50.14	4.45	0.50	0.00	0.44	0.23	0.00	2.79	0.48

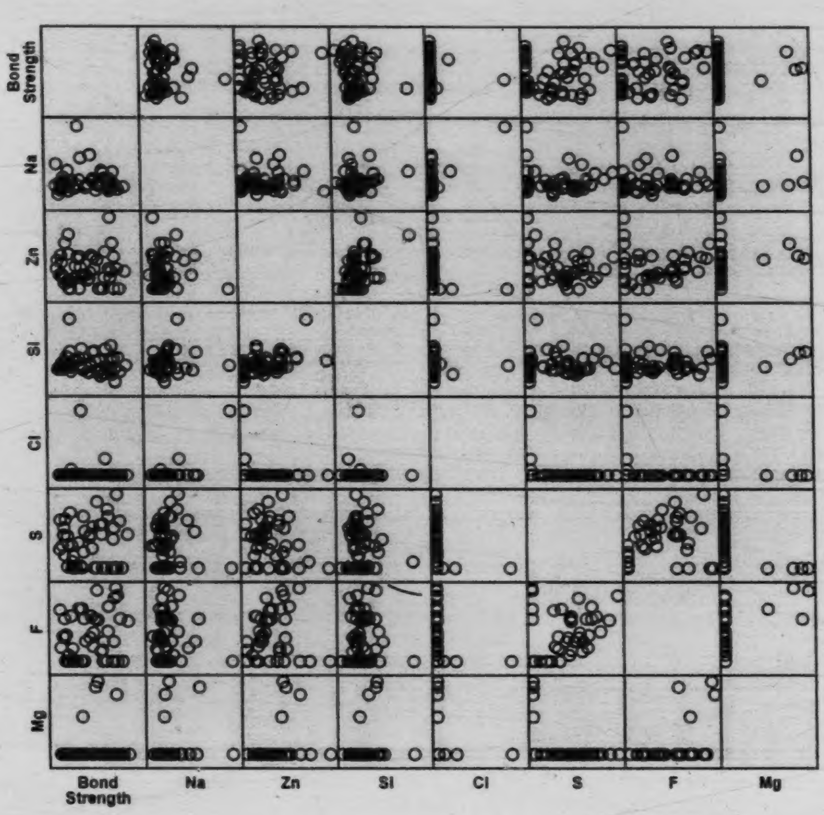
Appendix J: Statistics to accompany Chapter 3

Matrix of Scatter plots between bond strength and Ca, P, O, N, C, CO_3, and CO_3/P ratio



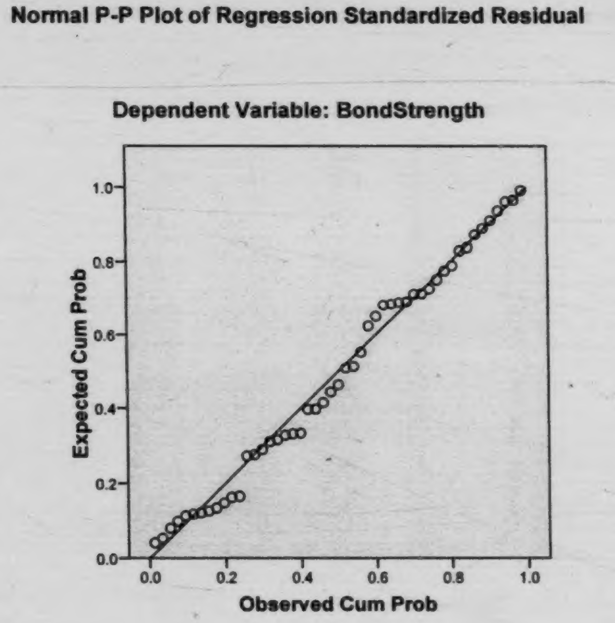
Matrix plot showed a strong positive correlation between Ca, P, and O.
Matrix plot also showed a strong positive correlation between C and N.
Ca, P and O however, are negatively correlated to C and N.
No strong relationship was found between the elements and bond strength.

Matrix of scatter plots between bond strength and Na, Zn, Si, Cl, S, F and Mg

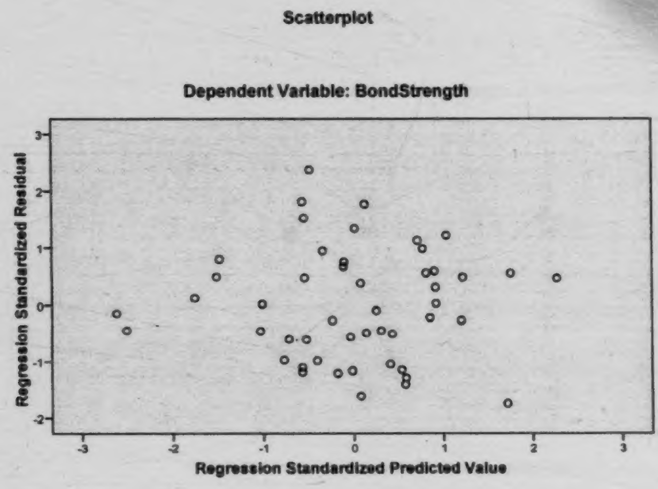


No strong relationship was found between any of the elements or between the elements and the bond strength

The plot below indicated no serious departures from the normality assumption



The plot of standardized residuals versus standardized predicted values showed a fairly constant spread of residuals indicating equal variances



Eleven multiple linear regression models were generated using backward multiple linear regressions. The explanatory variables were Ca, P, O, CO3, CO3/P, C, N, Na, Zn, Si, S, and F. The response variable was bond strength. Mg and Cl were not entered as explanatory variables as only few enamel samples contained these elements.

The model summary showed that when 12 explanatory variables were included in the model (model 1) 33.3% of the variation in bond strength was explained by these variables.

Model Summary(l)

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.577(a)	.333	.111	2.55963
2	.577(b)	.333	.135	2.52481
3	.577(c)	.333	.157	2.49220
4	.577(d)	.333	.179	2.46012
5	.576(e)	.331	.198	2.43142
6	.571(f)	.326	.211	2.41055
7	.553(g)	.306	.207	2.41697
8	.539(h)	.290	.208	2.41574
9	.518(i)	.269	.202	2.42422
10	.485(j)	.235	.184	2.45218
11	.434(k)	.188	.153	2.49814

a Predictors: (Constant), CO3/P, Zn, Na, S, Si, F, CO3, N, C, Ca, P, O
b Predictors: (Constant), CO3/P, Zn, S, Si, F, CO3, N, C, Ca, P, O
c Predictors: (Constant), CO3/P, Zn, S, Si, F, CO3, N, C, Ca, P
d Predictors: (Constant), CO3/P, Zn, S, Si, F, CO3, C, Ca, P
e Predictors: (Constant), Zn, S, Si, F, CO3, C, Ca, P
f Predictors: (Constant), S, Si, F, CO3, C, Ca, P
g Predictors: (Constant), Si, F, CO3, C, Ca, P
h Predictors: (Constant), Si, F, C, Ca, P
i Predictors: (Constant), Si, F, Ca, P
j Predictors: (Constant), Si, Ca, P
k Predictors: (Constant), Ca, P
l Dependent Variable: BondStrength

The ANOVA values for the11 regression model. Starting at model 4, the models became significant. But in each of the models, there were explanatory variables that were not significant. When all the insignificant variables were removed, model 11 was derived.

ANOVA(l)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	117.790	12	9.816	1.498	.170(a)
	Residual	235.861	36	6.552		
	Total	353.651	48			
2	Regression	117.789	11	10.708	1.680	.117(b)
	Residual	235.862	37	6.375		
	Total	353.651	48			
3	Regression	117.630	10	11.763	1.894	.077(c)
	Residual	236.021	38	6.211		
	Total	353.651	48			
4	Regression	117.616	9	13.068	2.159	.047(d)
	Residual	236.035	39	6.052		
	Total	353.651	48			
5	Regression	117.179	8	14.647	2.478	.028(e)
	Residual	236.472	40	5.912		
	Total	353.651	48			
6	Regression	115.410	7	16.487	2.837	.017(f)
	Residual	238.241	41	5.811		
	Total	353.651	48			
7	Regression	108.297	6	18.050	3.090	.013(g)
	Residual	245.354	42	5.842		
	Total	353.651	48			
8	Regression	102.711	5	20.542	3.520	.009(h)
	Residual	250.940	43	5.836		
	Total	353.651	48			
9	Regression	95.069	4	23.767	4.044	.007(i)
	Residual	258.582	44	5.877		
	Total	353.651	48			
10	Regression	83.057	3	27.686	4.604	.007(j)
	Residual	270.594	45	6.013		
	Total	353.651	48			
11	Regression	66.578	2	33.289	5.334	.008(k)
	Residual	287.073	46	6.241		
	Total	353.651	48			

a Predictors: (Constant), CO3/P, Zn, Na, S, Si, F, CO3, N, C, Ca, P, O
b Predictors: (Constant), CO3/P, Zn, S, Si, F, CO3, N, C, Ca, P, O

c Predictors: (Constant), CO3/P, Zn, S, Si, F, CO3, N, C, Ca, P
d Predictors: (Constant), CO3/P, Zn, S, Si, F, CO3, C, Ca, P
e Predictors: (Constant), Zn, S, Si, F, CO3, C, Ca, P
f Predictors: (Constant), S, Si, F, CO3, C, Ca, P
g Predictors: (Constant), Si, F, CO3, C, Ca, P
h Predictors: (Constant), Si, F, C, Ca, P
i Predictors: (Constant), Si, F, Ca, P
j Predictors: (Constant), Si, Ca, P
k Predictors: (Constant), Ca, P
l Dependent Variable: BondStrength

When each of the non-significant explanatory variables was removed, model 11 was derived. In model 11, only Ca, and P were significant predictors of bond strength. However, for Ca and P, the tolerance values were close to 0 (0.037) and the variance inflation factors (VIF of 27.326) were high. This indicated a high multi-colinearity and instability of the regression model. Therefore, Ca and P were run independently of each other in simple linear regression.

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	21.052	204.573		.103	.919	-393.841	435.945		
	Na	-.029	3.951	-.003	-.007	.994	-8.042	7.984	.095	10.532
	Zn	1.779	4.294	.084	.414	.681	-6.931	10.488	.454	2.204
	O	.091	2.022	.157	.045	.964	-4.009	4.191	.002	652.744
	N	.112	2.087	.072	.053	.958	-4.122	4.345	.010	98.000
	Ca	-3.210	2.283	-2.658	-1.406	.168	-7.840	1.420	.005	192.934
	C	-.219	2.050	-.586	-.107	.916	-4.377	3.939	.001	1625.434
	P	3.488	2.524	2.004	1.382	.176	-1.631	8.606	.009	113.560
	Si	-2.672	2.554	-.331	-1.046	.302	-7.852	2.507	.185	5.406
	S	-1.855	2.434	-.157	-.762	.451	-6.791	3.081	.435	2.300
	F	3.216	3.993	.219	.805	.426	-4.883	11.314	.251	3.987
	CO3	-.449	.407	-.239	-1.103	.277	-1.274	.377	.395	2.534
	CO3/P	.296	1.262	.066	.234	.816	-2.265	2.856	.231	4.323
2	(Constant)	19.643	69.554		.282	.779	-121.286	160.572		
	Zn	1.793	3.791	.084	.473	.639	-5.889	9.474	.567	1.765
	O	.105	.665	.181	.158	.875	-1.243	1.453	.014	72.673
	N	.126	.780	.081	.161	.873	-1.454	1.706	.071	14.055
	Ca	-3.195	1.111	-2.646	-2.876	.007	-5.446	-.944	.021	46.958
	C	-.205	.713	-.549	-.287	.775	-1.650	1.240	.005	202.138
	P	3.501	1.698	2.012	2.062	.046	.060	6.942	.019	52.845

	Si	-2.660	1.861	-.330	-1.429	.161	-6.431	1.112	.339	2.952
	S	-1.847	2.170	-.157	-.851	.400	-6.245	2.550	.532	1.880
	F	3.236	2.882	.220	1.123	.269	-2.603	9.075	.469	2.134
	CO3	-.450	.386	-.239	-1.166	.251	-1.231	.332	.428	2.338
	CO3/P	.298	1.204	.067	.248	.806	-2.141	2.737	.247	4.041
3	(Constant)	30.277	17.127		1.768	.085	-4.394	64.949		
	Zn	1.796	3.742	.085	.480	.634	-5.779	9.371	.567	1.765
	N	.017	.365	.011	.047	.962	-.721	.756	.317	3.156
	Ca	-3.287	.935	-2.722	-3.516	.001	-5.179	-1.395	.029	34.125
	C	-.312	.214	-.836	-1.462	.152	-.745	.120	.054	18.594
	P	3.363	1.436	1.933	2.341	.025	.455	6.271	.026	38.797
	Si	-2.802	1.607	-.347	-1.744	.089	-6.055	.451	.443	2.258
	S	-1.900	2.117	-.161	-.898	.375	-6.186	2.385	.545	1.835
	F	3.122	2.754	.212	1.134	.264	-2.452	8.696	.500	2.000
	CO3	-.454	.380	-.241	-1.194	.240	-1.223	.315	.429	2.329
	CO3/P	.318	1.182	.071	.269	.789	-2.075	2.711	.250	3.997
4	(Constant)	30.663	14.875		2.061	.046	.576	60.750		
	Zn	1.782	3.682	.084	.484	.631	-5.665	9.228	.570	1.753
	Ca	-3.296	.902	-2.730	-3.655	.001	-5.121	-1.472	.031	32.596
	C	-.315	.203	-.843	-1.554	.128	-.725	.095	.058	17.198
	P	3.354	1.405	1.927	2.387	.022	.512	6.196	.026	38.102
	Si	-2.835	1.431	-.351	-1.982	.055	-5.729	.059	.544	1.837
	S	-1.950	1.810	-.165	-1.077	.288	-5.612	1.712	.726	1.378
	F	3.100	2.679	.211	1.157	.254	-2.318	8.518	.515	1.942
	CO3	-.451	.372	-.240	-1.213	.232	-1.204	.301	.436	2.291
	CO3/P	.305	1.133	.068	.269	.790	-1.988	2.597	.265	3.771
5	(Constant)	31.312	14.506		2.158	.037	1.993	60.630		
	Zn	1.958	3.580	.092	.547	.587	-5.277	9.194	.589	1.697
	Ca	-3.349	.870	-2.773	-3.848	.000	-5.108	-1.590	.032	31.073

	C	-.319	.200	-.855	-1.599	.118	-.723	.084	.059	17.093
	P	3.336	1.387	1.917	2.405	.021	.532	6.139	.026	38.014
	Si	-2.880	1.404	-.357	-2.051	.047	-5.718	-.042	.552	1.811
	S	-1.942	1.789	-.165	-1.086	.284	-5.558	1.674	.726	1.377
	F	3.173	2.634	.216	1.205	.235	-2.150	8.495	.520	1.922
	CO3	-.399	.313	-.212	-1.276	.209	-1.030	.233	.604	1.656
6	(Constant)	28.820	13.654		2.111	.041	1.244	56.396		
	Ca	-3.319	.861	-2.749	-3.854	.000	-5.058	-1.580	.032	30.951
	C	-.288	.190	-.770	-1.518	.137	-.671	.095	.064	15.675
	P	3.475	1.352	1.997	2.571	.014	.745	6.205	.027	36.729
	Si	-2.474	1.182	-.307	-2.094	.042	-4.860	-.088	.766	1.305
	S	-1.962	1.773	-.166	-1.106	.275	-5.543	1.619	.726	1.377
	F	3.466	2.557	.236	1.356	.183	-1.697	8.629	.543	1.843
	CO3	-.339	.291	-.181	-1.168	.250	-.926	.248	.686	1.457
7	(Constant)	25.887	13.430		1.927	.061	-1.217	52.990		
	Ca	-3.165	.852	-2.622	-3.715	.001	-4.885	-1.446	.033	30.148
	C	-.253	.187	-.677	-1.349	.184	-.631	.125	.066	15.244
	P	3.389	1.353	1.948	2.505	.016	.658	6.120	.027	36.607
	Si	-2.275	1.171	-.282	-1.943	.059	-4.638	.088	.785	1.274
	F	2.654	2.456	.181	1.081	.286	-2.301	7.610	.591	1.691
	CO3	-.280	.286	-.149	-.978	.334	-.858	.298	.711	1.407
8	(Constant)	21.445	12.632		1.698	.097	-4.031	46.920		
	Ca	-3.080	.847	-2.551	-3.635	.001	-4.788	-1.371	.034	29.828
	C	-.208	.182	-.556	-1.144	.259	-.574	.158	.070	14.317
	P	3.569	1.340	2.051	2.664	.011	.867	6.271	.028	35.929
	Si	-2.242	1.170	-.278	-1.917	.062	-4.601	.117	.785	1.273
	F	2.968	2.433	.202	1.220	.229	-1.940	7.875	.602	1.662
9	(Constant)	7.047	1.128		6.247	.000	4.773	9.320		
	Ca	-2.922	.839	-2.420	-3.484	.001	-4.613	-1.232	.034	29.044
	P	4.226	1.215	2.428	3.478	.001	1.777	6.674	.034	29.342

	Si	-1.714	1.079	-.212	-1.589	.119	-3.888	.460	.930	1.075
	F	3.440	2.406	.234	1.430	.160	-1.409	8.290	.619	1.614
10	(Constant)	6.718	1.117		6.013	.000	4.468	8.968		
	Ca	-2.842	.847	-2.354	-3.357	.002	-4.547	-1.137	.035	28.914
	P	4.364	1.225	2.508	3.562	.001	1.896	6.831	.034	29.157
	Si	-1.803	1.089	-.223	-1.655	.105	-3.997	.391	.933	1.072
11	(Constant)	5.809	.991		5.861	.000	3.814	7.804		
	Ca	-2.514	.838	-2.082	-2.998	.004	-4.202	-.826	.037	27.326
	P	3.855	1.208	2.216	3.191	.003	1.423	6.288	.037	27.326

a Dependent Variable: BondStrength

The excluded variables in each of the models

Excluded Variables(k)

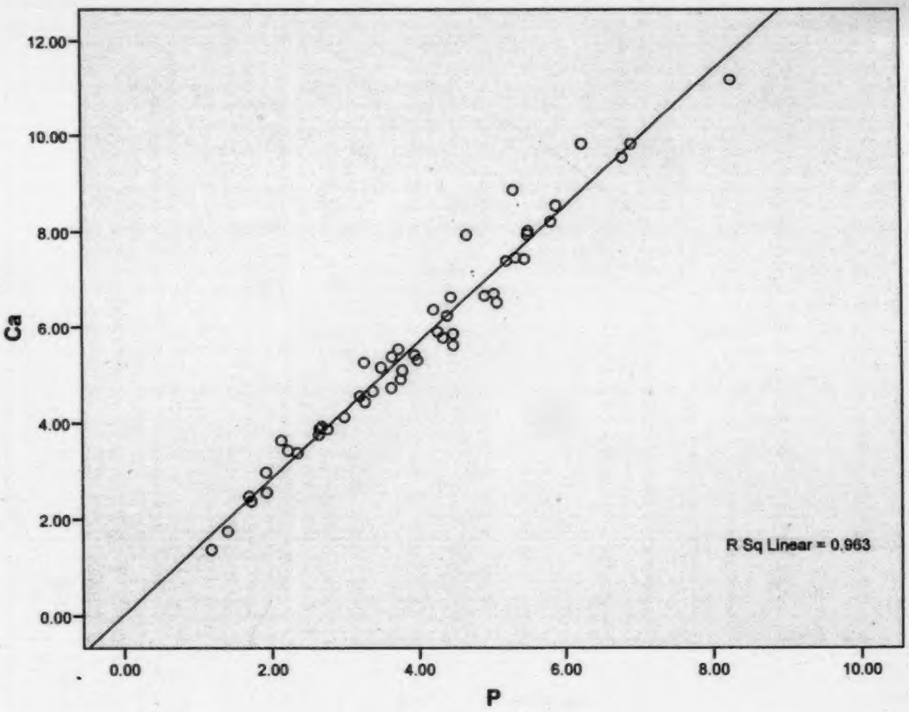
Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics		
						Tolerance	VIF	Minimal Tolerance
2	Na	-.003(a)	-.007	.994	-.001	.095	10.532	.001
3	Na	-.022(b)	-.151	.881	-.025	.853	1.173	.025
	O	.181(b)	.158	.875	.026	.014	72.673	.005
4	Na	-.022(c)	-.152	.880	-.025	.854	1.171	.026
	O	.018(c)	.034	.973	.006	.061	16.316	.022
	N	.011(c)	.047	.962	.008	.317	3.156	.026
5	Na	-.028(d)	-.204	.840	-.033	.888	1.126	.026
	O	.052(d)	.103	.919	.016	.066	15.231	.022
	N	-.004(d)	-.017	.986	-.003	.336	2.977	.026
	CO3/P	.068(d)	.269	.790	.043	.265	3.771	.026
6	Na	-.042(e)	-.313	.756	-.049	.927	1.079	.027
	O	.084(e)	.168	.868	.027	.067	15.014	.024
	N	-.019(e)	-.084	.933	-.013	.341	2.932	.027
	CO3/P	.090(e)	.365	.717	.058	.274	3.650	.027
	Zn	.092(e)	.547	.587	.086	.589	1.697	.026

7	Na	-.038(f)	-.279	.782	-.044	.928	1.078	.027
	O	-.120(f)	-.256	.799	-.040	.078	12.878	.025
	N	.089(f)	.458	.649	.071	.447	2.237	.027
	CO3/P	.087(f)	.350	.728	.055	.274	3.650	.027
	Zn	.096(f)	.568	.573	.088	.589	1.697	.026
	S	-.166(f)	-1.106	.275	-.170	.726	1.377	.027
8	Na	-.037(g)	-.276	.784	-.043	.928	1.078	.027
	O	-.105(g)	-.226	.822	-.035	.078	12.866	.026
	N	.080(g)	.410	.684	.063	.448	2.232	.027
	CO3/P	-.057(g)	-.285	.777	-.044	.418	2.390	.028
	Zn	.029(g)	.186	.853	.029	.674	1.483	.027
	S	-.134(g)	-.902	.372	-.138	.752	1.330	.028
	CO3	-.149(g)	-.978	.334	-.149	.711	1.407	.027
9	Na	-.037(h)	-.270	.789	-.041	.928	1.078	.034
	O	.216(h)	.763	.450	.116	.210	4.758	.031
	N	.120(h)	.631	.531	.096	.467	2.140	.034
	CO3/P	-.017(h)	-.087	.931	-.013	.431	2.320	.033
	Zn	-.007(h)	-.047	.963	-.007	.703	1.422	.034
	S	-.111(h)	-.751	.457	-.114	.764	1.310	.033
	CO3	-.098(h)	-.659	.513	-.100	.757	1.322	.034
	C	-.556(h)	-1.144	.259	-.172	.070	14.317	.028
10	Na	-.066(i)	-.489	.627	-.074	.953	1.050	.034
	O	.299(i)	1.088	.283	.162	.224	4.464	.031
	N	.031(i)	.167	.868	.025	.515	1.943	.034
	CO3/P	.003(i)	.017	.986	.003	.433	2.307	.033
	Zn	.018(i)	.115	.909	.017	.713	1.403	.034
	S	-.035(i)	-.242	.810	-.036	.854	1.171	.033
	CO3	-.116(i)	-.773	.444	-.116	.762	1.312	.034
	C	-.657(i)	-1.364	.180	-.201	.072	13.905	.028
	F	.234(i)	1.430	.160	.211	.619	1.614	.034
11	Na	-.093(j)	-.683	.498	-.101	.968	1.033	.036
	O	.104(j)	.395	.695	.059	.258	3.869	.032

	N	.122(j)	.697	.489	.103	.580	1.724	.035
	CO3/P	-.005(j)	-.026	.979	-.004	.434	2.306	.035
	Zn	-.091(j)	-.649	.520	-.096	.909	1.101	.036
	S	-.007(j)	-.047	.963	-.007	.866	1.154	.036
	CO3	-.135(j)	-.888	.380	-.131	.767	1.303	.036
	C	-.276(j)	-.599	.552	-.089	.084	11.871	.028
	F	.249(j)	1.499	.141	.218	.622	1.609	.036
	Si	-.223(j)	-1.655	.105	-.240	.933	1.072	.034

a Predictors in the Model: (Constant), CO3/P, Zn, S, Si, F, CO3, N, C, Ca, P, O
b Predictors in the Model: (Constant), CO3/P, Zn, S, Si, F, CO3, N, C, Ca, P
c Predictors in the Model: (Constant), CO3/P, Zn, S, Si, F, CO3, C, Ca, P
d Predictors in the Model: (Constant), Zn, S, Si, F, CO3, C, Ca, P
e Predictors in the Model: (Constant), S, Si, F, CO3, C, Ca, P
f Predictors in the Model: (Constant), Si, F, CO3, C, Ca, P
g Predictors in the Model: (Constant), Si, F, C, Ca, P
h Predictors in the Model: (Constant), Si, F, Ca, P
i Predictors in the Model: (Constant), Si, Ca, P
j Predictors in the Model: (Constant), Ca, P
k Dependent Variable: BondStrength

Ca and P showing a strong positive correlation with R^2 of 96.3%



Ca and P were significantly (p<0.000) correlated

Correlations

		Ca	P
Pearson Correlation	Ca	1.000	.982
	P	.982	1.000
Sig. (1-tailed)	Ca	.	.000
	P	.000	.
N	Ca	49	49
	P	49	49

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.982[a]	.963	.963	.43459

[a] Predictors: (Constant), P

[b] Dependent Variable: Ca

ANOVA showed significant correlation between Ca and P. Therefore, Ca and P will be run separately in simple linear regression.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	233.688	1	233.688	1237.325	.000[a]
	Residual	8.877	47	.189		
	Total	242.565	48			

a. Predictors: (Constant), P

b. Dependent Variable: Ca

Descriptive statistics for the element Ca

Descriptive Statistics

	Mean	Std. Deviation	N
BondStrength	6.9347	2.71436	49
Ca	5.6853	2.24798	49

Calcium was able to predict 0.09% of the variation in bond strength

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.093[a]	.009	-.012	2.73125

a. Predictors: (Constant), Ca

b. Dependent Variable: BondStrength

Ca was not a significant predictor in bond strength (p=0.526), hence the null hypothesis of H_0: $\beta=0$ was not rejected.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.045	1	3.045	.408	.526[a]
	Residual	350.606	47	7.460		
	Total	353.651	48			

a. Predictors: (Constant), Ca

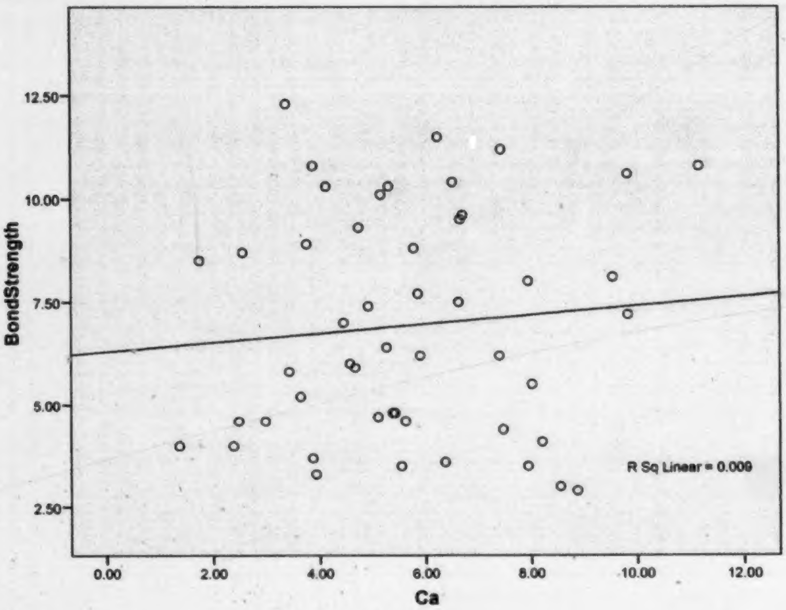
b. Dependent Variable: BondStrength

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	6.298	1.071		5.882	.000	4.144	8.452		
	Ca	.112	.175	.093	.639	.526	-.241	.465	1.000	1.000

a. Dependent Variable: BondStrength

The scatter plot of bond strength to Ca showed insignificant relationship between the two variables



Descriptive statistics for P

Descriptive Statistics

	Mean	Std. Deviation	N
BondStrength	6.9347	2.71436	49
P	3.9990	1.55989	49

P was able to predict 3.0% of the variation in bond strength

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.172[a]	.030	.009	2.70212

a. Predictors: (Constant), P

b. Dependent Variable: BondStrength

P was not a significant predictor in bond strength (p=0.237), hence the null hypothesis of *H*₀: β=0 was not rejected.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	10.482	1	10.482	1.436	.237[a]
	Residual	343.169	47	7.301		
	Total	353.651	48			

a. Predictors: (Constant), P

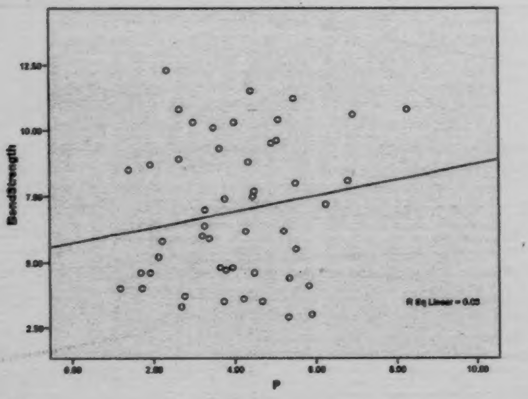
b. Dependent Variable: BondStrength

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	5.737	1.072		5.352	.000	3.581	7.893		
	P	.300	.250	.172	1.198	.237	-.203	.803	1.000	1.000

a. Dependent Variable: BondStrength

The scatter plot of bond strength to P showed insignificant relationship between the two variables



Descriptive statistics for CO_3

Descriptive Statistics

	Mean	Std. Deviation	N
BondStrength	6.9347	2.71436	49
CO3	3.1378	1.44500	49

When CO_3 was used a single predictor of bond strength, it explained 2.4% of the variation seen in bond strength.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.155[a]	.024	.003	2.70998

a. Predictors: (Constant), CO3

b. Dependent Variable: BondStrength

But the prediction of bond strength was not significant (p=0.288), therefore the null hypothesis of H_0: $\beta=0$ was not rejected.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	8.484	1	8.484	1.155	.288[a]
	Residual	345.167	47	7.344		
	Total	353.651	48			

a. Predictors: (Constant), CO3

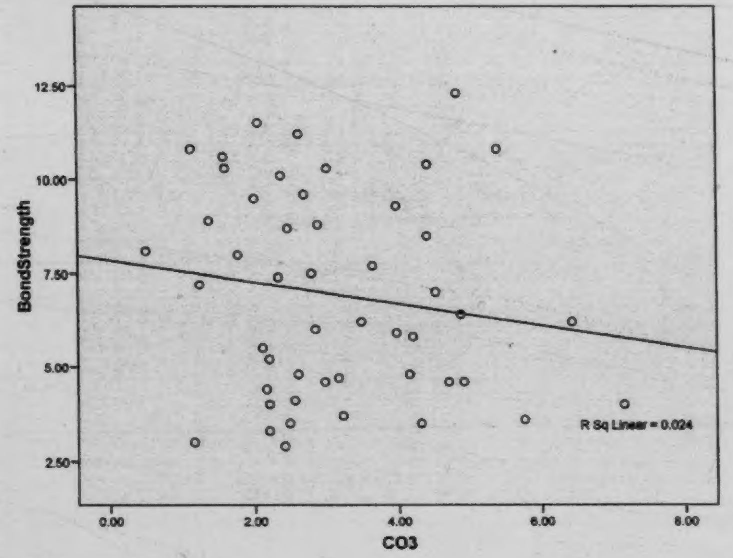
b. Dependent Variable: BondStrength

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	7.848	.933		8.407	.000	5.970	9.725		
	CO3	-.291	.271	-.155	-1.075	.288	-.836	.254	1.000	1.000

a. Dependent Variable: BondStrength

The scatter plot of bond strength to CO_3 showed no significant relationship between the two variables, but there appeared to be three outliers which were samples CH74-14B, EM6-24B and TU15-24B.



The three outlier samples were removed and the regression analysis performed again.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.032[a]	.001	-.022	2.74676

a. Predictors: (Constant), CO3

b. Dependent Variable: BondStrength

When the outliers were removed, carbonate was able to explain 0.1% of the variation in mean bond strength, but the contribution was not significant (p= 0.832)

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.345	1	.345	.046	.832[a]
	Residual	331.967	44	7.545		
	Total	332.312	45			

a. Predictors: (Constant), CO3

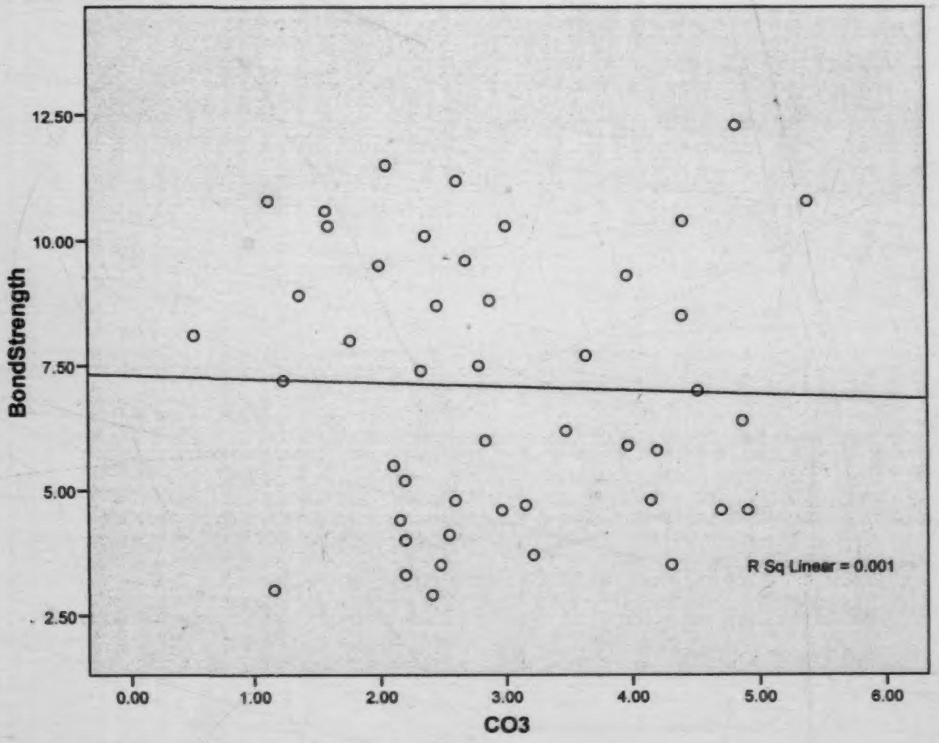
b. Dependent Variable: BondStrength

The null hypothesis of H_0: $\beta=0$ was not rejected. β has a negative value of -0.073 when outliers were removed as opposed to β = -0.291 when outliers were left in the analysis. The negative value indicated that as carbonate content increased, the bond strength decreased, but this relationship was not statistically significant (p=0.832).

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	7.301	1.080		6.761	.000
	CO3	-.073	.343	-.032	-.214	.832

a. Dependent Variable: BondStrength



Descriptive statistics for CO_3/P

Descriptive Statistics

	Mean	Std. Deviation	N
BondStrength	6.9347	2.71436	49
CO3/P	.8024	.60848	49

CO_3/P explained 0.05% of the variation noted in bond strength

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.074[a]	.005	-.016	2.73554

a. Predictors: (Constant), CO3/P

b. Dependent Variable: BondStrength

But the regression was not significant, p=0.613. Therefore the null hypothesis of H_0: $\beta=0$ was not rejected.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.942	1	1.942	.259	.613[a]
	Residual	351.709	47	7.483		
	Total	353.651	48			

a. Predictors: (Constant), CO3/P

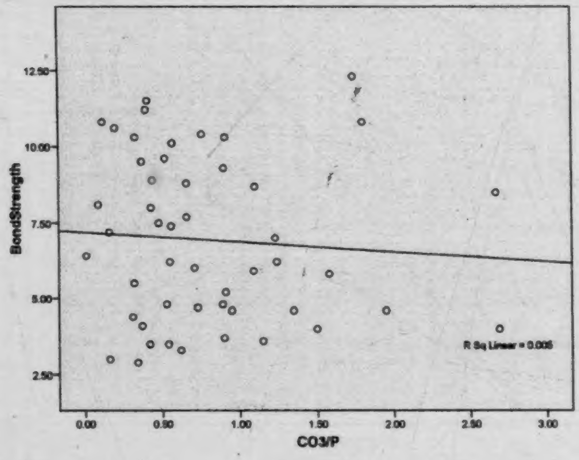
b. Dependent Variable: BondStrength

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	7.200	.651		11.059	.000	5.890	8.510		
	CO3/P	-.331	.649	-.074	-.509	.613	-1.636	.975	1.000	1.000

a. Dependent Variable: BondStrength

The scatter plot of bond strength to CO_3/P showed no significant relationship between the two variables



In summary, when all 12 explanatory variables were included in the regression model, 33.3% of the variation in the *in vitro* bond strength was explained, but the regression was not significant, p=0.170

Appendix K: Post hoc power analysis

Post hoc power analysis was performed based on the formula for multiple regression formula of:

$N = (L/f^2) + K + 1$, where $f^2 = R^2/1\text{-}R^2$

$R^2 = 0.3333$ with 12 explanatory variables

$f^2 = 0.333/\ 1\text{-}0.333 = 0.499$

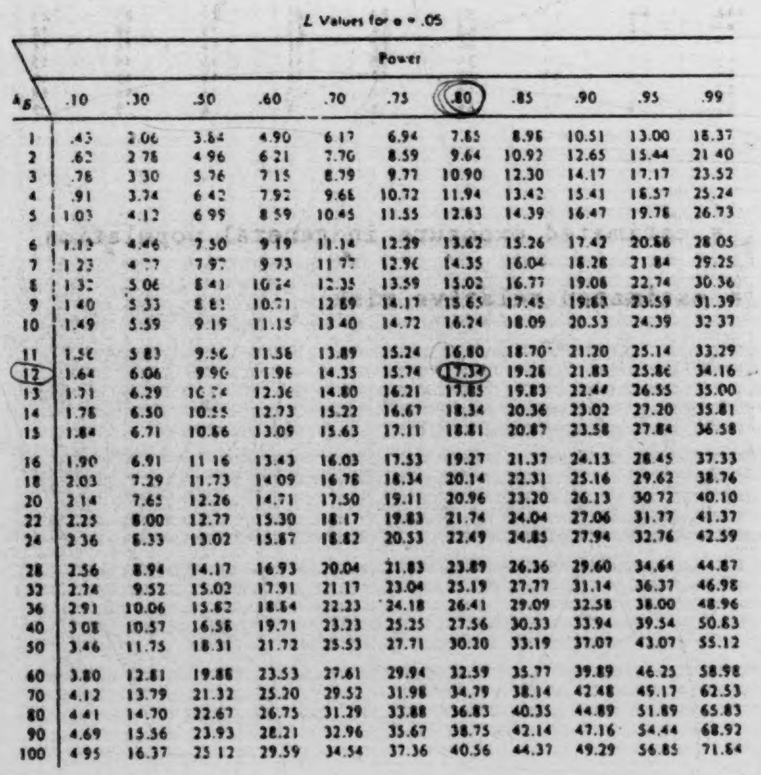
K = number of independent variables = 12

N = 49 patients included in the study

$$L = (N - 1 - K) \times f^2$$

$$L = (49 - 1\text{-}\ 12) \times 0.4999 = 17.973 \text{ for } \alpha = 0.05$$

Inserting the L value of 17.973 and a K_β value of 12, a power of 80% was derived



L Values for α = .05

	Power										
K_β	.10	.30	.50	.60	.70	.75	.80	.85	.90	.95	.99
1	.43	2.06	3.84	4.90	6.17	6.94	7.85	8.96	10.51	13.00	18.37
2	.62	2.78	4.96	6.21	7.70	8.59	9.64	10.92	12.65	15.44	21.40
3	.78	3.30	5.76	7.15	8.79	9.77	10.90	12.30	14.17	17.17	23.52
4	.91	3.74	6.42	7.92	9.68	10.72	11.94	13.42	15.41	18.57	25.24
5	1.03	4.12	6.99	8.59	10.45	11.55	12.83	14.39	16.47	19.78	26.73
6	1.13	4.46	7.50	9.19	11.14	12.29	13.62	15.26	17.42	20.86	28.05
7	1.23	4.77	7.97	9.73	11.77	12.96	14.35	16.04	18.28	21.84	29.25
8	1.32	5.06	8.41	10.24	12.35	13.59	15.02	16.77	19.08	22.74	30.36
9	1.40	5.33	8.81	10.71	12.89	14.17	15.65	17.45	19.83	23.59	31.39
10	1.49	5.59	9.19	11.15	13.40	14.72	16.24	18.09	20.53	24.39	32.37
11	1.56	5.83	9.56	11.58	13.89	15.24	16.80	18.70	21.20	25.14	33.29
12	1.64	6.06	9.90	11.98	14.35	15.74	17.34	19.28	21.83	25.86	34.16
13	1.71	6.29	10.24	12.36	14.80	16.21	17.85	19.83	22.44	26.55	35.00
14	1.78	6.50	10.55	12.73	15.22	16.67	18.34	20.36	23.02	27.20	35.81
15	1.84	6.71	10.86	13.09	15.63	17.11	18.81	20.87	23.58	27.84	36.58
16	1.90	6.91	11.16	13.43	16.03	17.53	19.27	21.37	24.13	28.45	37.33
18	2.03	7.29	11.73	14.09	16.78	18.34	20.14	22.31	25.16	29.62	38.76
20	2.14	7.65	12.26	14.71	17.50	19.11	20.96	23.20	26.13	30.72	40.10
22	2.25	8.00	12.77	15.30	18.17	19.83	21.74	24.04	27.06	31.77	41.37
24	2.36	8.33	13.02	15.87	18.82	20.53	22.49	24.85	27.94	32.76	42.59
28	2.56	8.94	14.17	16.93	20.04	21.83	23.89	26.36	29.60	34.64	44.87
32	2.74	9.52	15.02	17.91	21.17	23.04	25.19	27.77	31.14	36.37	46.98
36	2.91	10.06	15.82	18.84	22.23	24.18	26.41	29.09	32.58	38.00	48.96
40	3.08	10.57	16.58	19.71	23.23	25.25	27.56	30.33	33.94	39.54	50.83
50	3.46	11.75	18.31	21.72	25.53	27.71	30.20	33.19	37.07	43.07	55.12
60	3.80	12.81	19.88	23.53	27.61	29.94	32.59	35.77	39.89	46.25	58.98
70	4.12	13.79	21.32	25.20	29.52	31.98	34.79	38.14	42.48	49.17	62.53
80	4.41	14.70	22.67	26.75	31.29	33.88	36.83	40.35	44.89	51.89	65.83
90	4.69	15.56	23.93	28.21	32.96	35.67	38.75	42.14	47.16	54.44	68.92
100	4.95	16.37	25.12	29.59	34.54	37.36	40.56	44.37	49.29	56.85	71.64

Appendix L: Raw data showing the inter-relationship between qualitative etching pattern and enamel chemical composition

Samples	Etching Pattern 1st attempt classification	Etching Pattern 2nd attempt classification	Na	Zn	O	N	Ca	C	P	Si	Cl	S	F	Mg	CO_3	CO_3/P
AB11-14B	4	4	0.39	0.3	31.5	7.07	5.28	49.98	4.01	0.72	0	0	0.37	0.38	2.5	0.5
AH8-24B	1	1	0.21	0.25	28.09	5.48	5.92	54.28	4.46	0.66	0	0.3	0.36	0	3.01	0.57
AH9-24B	3	3	0.66	0.13	37.02	6.05	6.91	42.34	5.08	1.11	0	0	0.54	0.16	1.15	0.17
BE61-14B	4	4	0.31	0.27	28.98	7.43	3.04	56.84	2.31	0.61	0	0.2	0	0	5.77	2.28
BE63-14B	3	3	0.29	0.1	37.98	5.14	10	38.46	6.74	0.59	0	0.29	0.38	0	1.2	0.16
BR54-14B	3	4	0.35	0.05	26.81	8.46	3.05	57.6	2.36	0.63	0	0.45	0.24	0	1.21	0.33
CH71-24B	2	2	0.18	0	35.54	4.57	7.96	44.14	5.63	1.27	0	0.25	0.45	0	1.44	0.21
CH74-24B	2	2	0.18	0.24	28.42	8.06	2.79	57.49	1.92	0.65	0.02	0.23	0	0	11.1	5.24
DA32-14B	1	1	0.41	0.23	34.8	4.89	4.44	50.58	3.11	1.49	0.01	0.04	0	0	2.81	0.71
DE43-14B	1	1	0.44	0.04	33.44	5.95	5.19	49.55	3.89	0.93	0	0.34	0.24	0	2.5	0.55
DH6-24B	3	3	0.93	0.05	41.99	4.03	9.89	34.73	7.12	0.45	0	0.49	0.33	0	0.5	0.06
DO35-24B	1	1	0.44	0.12	28.95	9.67	4.21	53.09	3.08	0.45	0	0	0	0	3.33	0.93
EL11-14B	1	1	0.19	0.1	26.62	9.66	4.42	55.11	2.96	0.51	0	0.29	0.14	0	3.35	1.13
EM6--14B	1	1	0.33	0.2	31.63	7.38	4.82	51.53	3.39	0.7	0.01	0.02	0	0	6.97	1.54
FA91-24B	3	3	0.36	0.14	35.96	4.75	7.11	44.62	5.08	1.05	0	0.6	0.34	0	0	0
GE15-24B	4	4	0.29	0.22	23.93	10.7	1.91	61.14	1.17	0.39	0	0.19	0.08	0	4.44	2.85
HA88-24B	2	2	0.15	0	24.83	8.49	4.19	57.83	3.11	1.41	0	0	0	0	3.19	0.79
HE44-14B	3	3	0.18	0.24	26.54	7.25	1.7	61.94	1.43	0.26	0	0.21	0.25	0	4.25	2.55
HO48-14B	3	3	0.33	0	27.22	10.3	4.28	54.83	2.99	0	0	0	0	0	4.88	1.16
HU52-24B	4	4	0.27	0.07	38.03	5.5	9.83	38.93	6.25	0.28	0	0.36	0.36	0	1.24	0.16
HU54-24B	2	2	0.27	0.16	25.92	9.09	4.48	56.46	3.23	0.39	0	0	0	0	3.32	0.9
KH15-14B	2	2	0.29	0.02	36.74	4.72	8.34	42.51	6.12	0.4	0	0.39	0.48	0	0.56	0.08
MA119-14B	3	3	0.25	0.14	33.25	6.56	5.31	50.26	3.64	0.51	0.02	0.05	0	0	0	0
MA122-14B	3	3	0.38	0.28	33.22	7.61	7.4	45.36	5.34	0.41	0	0	0	0	2.24	0.36
MO55-24B	4	4	0.31	0.14	34.45	5.84	6.61	46.46	4.88	0.37	0	0.65	0.3	0	2.98	0.49

MO57-14B	4	4	0.44	0.11	39.77	3.65	9.17	38.18	6.59	0.59	0	1.11	0.4	0	2	0.26
MU35-24B	3	4	1.29	0	23.6	8.13	3.43	59.15	2.49	0.65	1.26	0	0	0	3.33	1.2
NG14-14B	3	3	0.27	0.14	29.31	6.96	3.38	56.39	2.35	0.54	0	0.36	0.32	0	4.83	1.74
NU4-24B	2	1	0.52	0	31.29	8.15	3.24	53.11	2.62	0.48	0	0.17	0.42	0	2.62	0.9
PA62-14B	3	3	0.5	0.38	39.51	4.17	9.47	37.51	6.83	0.78	0	0.33	0.52	0	2.26	0.28
PA66-24B	4	4	0.6	0.25	35.83	5.86	7.17	43.93	4.95	0.74	0	0	0.39	0.28	2.77	0.53
PE45-14B	3	3	0.09	0.06	22.26	10.2	2.15	63.11	1.34	0.45	0	0.15	0.15	0	5.23	3.17
RE35-14B	3	3	0.36	0.39	36.7	4.3	8.59	42.53	5.93	0.4	0	0.43	0.37	0	1.87	0.28
RE46-14B	3	3	0.28	0	29.38	7.62	5.18	53.22	3.74	0.58	0	0	0	0	2.98	0.68
RE50-24B	4	4	0.36	0.02	33.44	5.89	4.93	50.97	3.41	0.73	0	0.08	0.18	0	2.44	0.62
RI19-24B	4	4	0.2	0.11	30.44	5.75	3.28	57	2.49	0.41	0	0	0.22	0.09	3.47	1.75
RO62-14B	1	2	0.46	0.31	39.98	3.1	11.3	36.52	7.19	0.21	0	0.5	0.47	0	1.2	0.17
SI34-14B	3	3	0.46	0.07	38.14	4.35	8.1	41.39	5.61	0.92	0	0.5	0.46	0	0.63	0.09
SM17-24	4	4	0.2	0.3	30.49	6.58	6.81	49.57	4.89	0.51	0	0.4	0.24	0	2.25	0.38
SM19-24B	2	2	0.28	0.08	30.3	6.39	5.72	51.46	4.01	0.99	0	0.51	0.27	0	0.27	0.06
TA17-14B	4	4	0.3	0.3	30.61	8.5	5.68	50.03	3.91	0.66	0	0	0	0	2.63	0.54
TI11-24B	1	1	0.45	0.08	23.64	6.23	3.64	63.16	2.13	0.66	0	0	0	0	2.2	0.91
TO11-24B	2	2	0.32	0.02	25.34	7.31	3.3	60.8	2.16	0.33	0	0.18	0.24	0	2.73	1
TU15-14B	3	3	0.27	0.23	34.19	5.73	5.91	48.9	3.98	0.52	0	0.25	0	0	5.9	1.23
WE17-24B	2	2	0.18	0.12	28.19	7.66	2.91	57.08	2.51	0.35	0	0.53	0.47	0	3.29	1.5
WE24-24B	1	1	0.27	0.24	28.86	8.95	5.35	51.3	3.63	0.71	0	0.48	0.2	0	2.48	0.59
WI48-24B	1	1	0.45	0	24.65	10.8	3.12	58.75	2.16	0.12	0	0	0	0	3.01	1.11
YU5-24B	3	3	0.59	0	27.16	8.57	5.42	52.87	4.24	0.63	0.52	0	0	0	2.62	0.51
ZH4-24B	3	3	0.14	0.15	23.39	6.61	4.2	61.13	3.05	0.81	0	0.35	0.18	0	2.05	0.49

Appendix M: Raw data showing the relationship between qualitative acid etching pattern and *in vitro* shear bond strength

Samples	Etching Pattern 1st attempt classification	Bond Strength (MPa)
AB11-14B	4	10.4
AH8-24B	1	10.3
AH9-24B	3	10.1
BE61-14B	4	3
BE63-14B	3	6.2
BR54-14B	3	5.2
CH71-24B	2	3.5
CH74-24B	2	11.5
DA32-14B	1	4.1
DE43-14B	1	6.4
DH6-24B	3	8.9
DO35-24B	1	4
EL11-14B	1	9.3
EM6--14B	1	7
FA91-24B	3	10.3
GE15-24B	4	7.4
HA88-24B	2	8.7
HE44-14B	3	3.7
HO48-14B	3	3.3
HU52-24B	4	8
HU54-24B	2	4.6
KH15-14B	2	4
MA119-14B	3	8.1
MA122-14B	3	8.5
MO55-24B	4	10.8
MO57-14B	4	5.8
MU35-24B	3	8.8
NG14-14B	3	6
NU4-24B	2	3.5
PA62-14B	3	5.5
PA66-24B	4	9.6
PE45-14B	3	4.6
RE35-14B	3	11.2
RE46-14B	3	12.3
RE50-24B	4	3.6
RI19-24B	4	4.4
RO62-14B	1	4.8
SI34-14B	3	7.5
SM17-24	4	10.6
SM19-24B	2	4.8
TA17-14B	4	2.9

TI11-24B	1	4.6
TO11-24B	2	7.2
TU15-14B	3	10.8
WE17-24B	2	6.2
WE24-24B	1	4.7
WI48-24B	1	7.7
YU5-24B	3	9.5
ZH4-24B	3	5.9

Appendix N: Statistics to accompany Chapter 4

The intra-rater reliability of the two attempts at identifying the qualitative enamel acid etching pattern at an interval of 3 months apart showed a highly significant intra-rater reliability of 0.946 with a p-value of <0.0001.

Correlations

			Etching Pattern	Etching Pattern2
Kendall's tau_b	Etching Pattern	Correlation Coefficient	1.000	.946**
		Sig. (2-tailed)	.	.000
		N	49	49
	EtchingPattern2	Correlation Coefficient	.946**	1.000
		Sig. (2-tailed)	.000	.
		N	49	49
Spearman's rho	Etching Pattern	Correlation Coefficient	1.000	.967**
		Sig. (2-tailed)	.	.000
		N	49	49
	EtchingPattern2	Correlation Coefficient	.967**	1.000
		Sig. (2-tailed)	.000	.
		N	49	49

**. Correlation is significant at the 0.01 level (2-tailed).

McNemar test showing the reliability in identifying etching pattern based on two separate attempts

Etching Pattern * EtchingPattern2 Crosstabulation

Count

		EtchingPattern2				
		1	2	3	4	Total
Etching Pattern	1	9	1	0	0	10
	2	1	8	0	0	9
	3	0	0	17	2	19
	4	0	0	0	11	11
Total		10	9	17	13	49

The McNemar test with a p value of 0.368 indicated that the disagreement in identifying etching pattern was likely to occur equally with all four etching patterns

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
McNemar-Bowker Test	2.000	2	.368
N of Valid Cases	49		

Ordinal regression showed that the chemical composition of all the elements of the buccal surface of maxillary first premolar explained 20.8% of the observed variation observed in enamel acid etching pattern

Pseudo R-Square

Cox and Snell	.427
Nagelkerke	.459
McFadden	.208

Link function: Logit.

The regression between enamel surface chemical composition and qualitative etching pattern was, however not significant with a p-value of 0.3999 based on Wilk's Lambda.

Multivariate Tests[d]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	Pillai's Trace	1.000	1E+006[b]	14.000	32.000	.000	1.000	1.41E+009	1.000
	Wilks' Lambda	.000	1E+008[b]	14.000	32.000	.000	1.000	1.41E+009	1.000
	Hotelling's Trace	4E+007	1E+008[b]	14.000	32.000	.000	1.000	1.41E+009	1.000
	Roy's Largest Root	4E+007	1E+008[b]	14.000	32.000	.000	1.000	1.41E+009	1.000
EtchingPattern	Pillai's Trace	.917	1.069	42.000	102.000	.384	.306	44.915	.905
	Wilks' Lambda	.322	1.060	42.000	95.693	.399	.315	43.919	.891
	Hotelling's Trace	1.432	1.046	42.000	92.000	.420	.323	43.929	.887
	Roy's Largest Root	.757	1.839[c]	14.000	34.000	.073	.431	25.740	.814

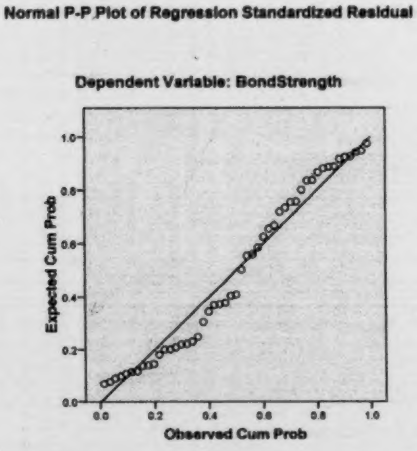
a. Computed using alpha = .05

b. Exact statistic

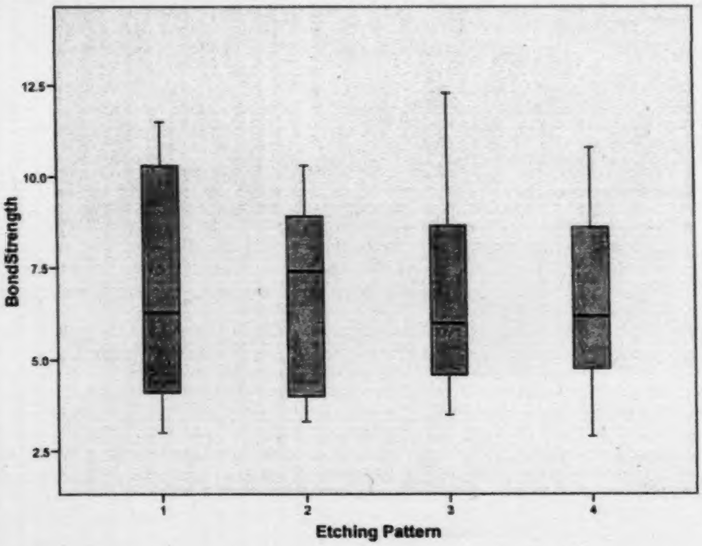
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

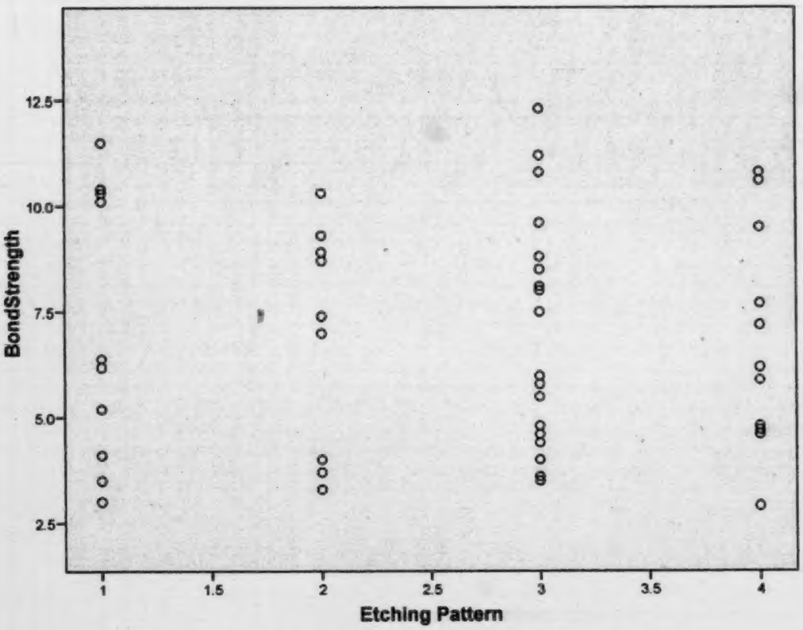
d. Design: Intercept+EtchingPattern

The plot below showed normality of bond strength



The boxplot and scatter plot below showed the outcome variable, bond strength, to have approximately equal variances





Descriptive statistics for the relationship between qualitative etching pattern and mean shear bond strength

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
TypeI	10	3.0	11.5	7.070	3.2173
TypeII	9	3.3	10.3	6.667	2.7879
TypeIII	19	3.5	12.3	7.063	2.6736
TypeIV	11	2.9	10.8	6.809	2.6140
BondStrength	49	2.9	12.3	6.935	2.7144
Valid N (listwise)	9				

ANOVA revealed no statistically significant difference ($p = 0.997$) in the mean shear bond strength regardless of the qualitative etching pattern

ANOVA

BondStrength

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.362	3	.121	.015	.997
Within Groups	353.289	45	7.851		
Total	353.651	48			

Linear regression showed an R^2 of 0.1%. That is, only 0.1% of the variation in bond strength was explained by the qualitative etching pattern

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.031[a]	.001	-.020	2.7418

a. Predictors: (Constant), Etching Pattern

b. Dependent Variable: BondStrength

The regression was not statistically significant with a p-value of 0.832

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.342	1	.342	.046	.832[a]
	Residual	353.309	47	7.517		
	Total	353.651	48			

[a] Predictors: (Constant), Etching Pattern

[b] Dependent Variable: BondStrength

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	7.146	1.063		6.724	.000
	Etching Pattern	-.080	.375	-.031	-.213	.832

[a] Dependent Variable: BondStrength